Ishan Patro · Pankaj Seth
Nisha Patro · Prakash Narain Tandon *Editors*

The Biology of Glial Cells: Recent Advances

Springer

The Biology of Glial Cells: Recent Advances

Ishan Patro • Pankaj Seth • Nisha Patro •
Prakash Narain Tandon
Editors

The Biology of Glial Cells: Recent Advances

Springer

Editors
Ishan Patro
School of Studies in Neuroscience
Jiwaji University
Gwalior, Madhya Pradesh, India

Nisha Patro
School of Studies in Neuroscience
Jiwaji University
Gwalior, Madhya Pradesh, India

Pankaj Seth
Cellular and Molecular Neurosciences
National Brain Research Centre
Manesar, Haryana, India

Prakash Narain Tandon
National Brain Research Centre
Manesar, Haryana, India

ISBN 978-981-16-8312-1 ISBN 978-981-16-8313-8 (eBook)
https://doi.org/10.1007/978-981-16-8313-8

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Dedicated to Our Students

Foreword

As a cell biologist who spent 25 years working on deciphering the detailed mechanisms that a cell uses to replicate its DNA, I greatly admire the courage of the many researchers around the globe who are attempting to understand the brain. The model system that I focused on was from bacteriophage T4. This virus infects the *E. coli* bacterium; and its close relatives are abundant in the world's oceans, responsible for a great deal of the nutrient recycling that feeds other organisms (Suttle, 2007). The mystery that I had to unravel was the detailed mechanism of a protein machine formed from 7 virus-encoded proteins (Alberts, 1984). This was a struggle for my laboratory; but it is nothing compared to the task of making sense of the human brain, which is composed of more than 100 billion neurons, each of which, on average, forms synapses with a thousand others in intricately patterned arrays. These nerve cells are supported in critical ways—many not yet understood—by the glia, the brain cells that outnumber the neurons two to one and are the focus of this monograph.

Science is a great international effort of the world's community of scientists, and brain science in particular is a field of tremendous challenge that will require the talents and resources of every nation for its success. India, a wonderful country that I have visited perhaps 20 times, has an immense scientific and technical potential. Its attention to neuroscience has been a relatively late development, in which one man—Prakash Narain Tandon—has played a major role.

I first met Prakash on my initial trip to India, in September 1993. The occasion was the first-ever meeting of all the world's science academies held in New Delhi. This meeting had been organized by the Indian National Science Academy (INSA), with Prakash serving as its president. Its focus was on world population problems, in order to produce a scientific view of population issues and layout a scientifically based agenda for achieving a sustainable future (Royal Society, 1994). The meeting was such a success that it led to the formation of a new permanent organization, the InterAcademy Panel on International Issues (IAP), established to enhance the important roles that science can play in humanity's future.

Prakash was chosen to present the views of science at the famous UN Conference on Population and Development that was held in Cairo in September 1994. In recognition of his immense talents and generous spirit, he was then chosen as one of the first co-chairs of the IAP (his partner was Sherwood Rowland, the Nobel Prize winner who was serving as the foreign secretary of the US National Academy of

Sciences, where I was president). Prakash and I then worked together intensely on international science issues for more than a decade, and we remain close friends in frequent contact.

It is very unusual for a distinguished neurosurgeon to become a leading scientist in his or her nation, much less to become a leader for science around the globe. This was only possible because Prakash is a very unique man. In fact, he reminds me very much of Charles Darwin, who wrote in his autobiography that "my mind seems to have become a kind of machine for grinding general laws out of large collections of facts." Now over 90, Prakash, mimicking Darwin's continued long productivity, continues to write prolifically on subjects that range from India's science history to neurosurgery, neuroscience, and education. His latest contribution, entitled "Glial Biology: A Historical Perspective," is Chap. 1 in this book. Also highly recommended is his recent autobiography, *Closed doors, open windows*. (Tandon, 2019).

As Prakash points out in his introductory chapter, once thought of simply as the "glue" that maintains neurons, glial cells (astrocytes, oligodendrocytes, and microglia) are now recognized to perform many different essential functions in the brain, playing informative roles in processes that range from neurogenesis and synaptogenesis to controlling the degeneration that occurs in aging and diseased brains. The progress made to date in understanding each of these functions is the subject of the 26 chapters that follow Chap. 1.

In closing, I want to commend Ishan Patro, Pankaj Seth, Nisha Patro, and Prakash Narain Tandon the editors of this important overview of the exciting and critical field of glial cell biology. The result of their efforts clearly highlights India's many contributions to the ongoing struggle to unravel the enormous complexities of brain science. This volume thus represents an important part of the great international effort to better understand fundamental human biology, a task that will be essential for improving the human condition.

References

Alberts BM (1984) The DNA enzymology of protein machines. Cold Spring Harb Symp Quant Biol 49:1–12

Royal Society (1994) Population—the complex reality. A report of the Population Summit of the world's scientific academies. Graham-Smith F (ed). Royal Society: London

Suttle CA (2007) Marine viruses—major players in the global ecosystem. Nat Rev Microbiol 5:801–812

Tandon PN (2019) Closed doors open windows: autobiography of a neurosurgeon chasing his dreams. Wolters Kluwer Health: India

Department of Biophysics and Biochemistry,
University of California, San Francisco (UCSF)
San Francisco, CA, USA

Bruce Alberts

Preface

More than a 100 years after Rudolf Ludwig Carl Virchow (1821–1902) discovered and designated these cells of the central nervous system as NeuroGlia, implying their function as a glue. Glia continued to be considered as "Subordinate," secondary, supporting cells of the neurons. This notwithstanding that Ramon Cajal (1852–1934), already in early years of the twentieth century, described the diverse morphology and functions of astrocytes. And soon after Rio del Hortega (1882–1945) identified the microglia and ascribed their possible functions and Wilder Penfield (1891–1976) working in his lab defined oligodendroglia. Nevertheless, the dominant role of neurons and their involvement in diverse functions of the brain preoccupied the attention of most neuroscientist—basic or clinical.

Surprisingly, it was only towards the end of the last century and more so during the last 2 to 3 decades that the researches of the neuroscientists established that glia are equal partners of neurons in their functions.

Fortunately, a number of neuroscientist in India also got interested in Glia Biology around this time. In addition to their individual efforts, they constituted a group with financial support of the Department of Biotechnology, Government of India, to promote this field of research. PNT was supported by the National Academy of Sciences (India) to work on this volume.

Consequent to their research output published as scientific papers, it was felt that with their help a comprehensive book could be published providing an updated account on all aspects of Glia Biology since there is a paucity of such literature. This book is the result of this collaborative effort of these scientists.

The book consists of 27 chapters dealing with basic biology of the glial cells—their structure, functions in health and disease, their interaction with neurons and each other. This is followed by their role in pathophysiology of diverse diseases of the nervous system and mental functions. Effort is made to point out the areas for future research and "tools" to do so. The book would be useful both for basic neuroscientists, students, and clinicians.

We are grateful to Prof. Bruce Alberts for his thought-provoking foreword. PNT is specially touched by his very laudatory reference to him. It is no doubt due to their very long friendship and his concern for advancement of Indian science to which he has contributed in a variety of ways.

We are thankful to Ms. Tiyasha Sarkar, Ms. Urmilla John, and Mr. Syed Mujtaba for their help in the editorial process and especially to Dr. Urmishree Bedamata for language corrections in several of the chapters.

We wish to place in record the support rendered by the publication team of Springer Nature.

Gwalior, India — Ishan Patro
Manesar, India — Pankaj Seth
Gwalior, India — Nisha Patro
Manesar, India — Prakash Narain Tandon

About the Book

Glial cells were identified by Virchow in 1854 (neuroglia). Cajal in 1897 elaborated the most common of these—the astrocytes—their detailed histology and possible functions. His pupil, del Rio Hortega, independently described the microglia and along with Penfield elaborated the oligodendroglia (1924). Preoccupied with the study of the neurons, for nearly a century not much attention was paid to the glial cells—generally considered as supporting cells of the "masters"—neurons. However, glial cells gathered the due attention during the past 2 to 3 decades.

Enormous information has thus accumulated in recent years on the structure and functions of glial cells, their cross-talks among themselves and with the neurons. These discoveries have great significance for our understanding of the complex function of the central nervous system in health and disease. They are now regarded to be equally important cell type of the brain, as neurons. Recent studies have established glial cells to be indispensable for neural maturation, maintenance, and function. These advances in our understanding of role of glial cells have designated glial neurobiology, as a rapidly advancing field.

Neuronal health and functions are regulated by the glial cells, and hence it necessitates compilation of recent advances in our understanding of the glial cell functions both in physiology and pathophysiology. The proposed book offers a compilation of recent research outcomes in understanding the role of glial cells (astrocytes, microglia, oligodendroglia, satellite cells, and the Schwann cells) in health and disease. The chapters in this book are carefully chosen to cover important aspects of glia biology, their role in regulation of neuronal functions, and their cross-talk during health and disease.

This book will be distinct from other materials available for the readers due to its content and updated knowledge. The other attractive features of this compilation would be a comprehensive, current yet concise write-up on important aspects of glial biology—a subject on which still there is a paucity of such reference-cum-textbooks.

This has prompted us to venture producing this book with the participation of 26 senior neuroscientists (and their groups), each a distinguished scientist with personal contributions in the field have joined us in this venture.

Contents

About the Editors

Ishan Patro is Professor of Zoology/Neuroscience at Jiwaji University, Gwalior, and Former Vice Chancellor of Ravenshaw University, Cuttack. He holds M.Phil. and Ph.D. degrees of Kurukshetra University, Kurukshetra. He had his Post-Doctoral at MRC Neurochemical Pathology Unit, Newcastle upon Tyne, UK, and Dept. of Anatomy, University of Cologne, Germany. He has 32 years of teaching and 40 years of research experience. He is elected Fellow of the National Academy of Sciences (India), Indian Academy of Neurosciences, Collegium Internationale Neuropsychopharmalogicum, and Honorary National Fellow of The Zoological Society, Kolkata. He has several awards and honors to his credit including the B.K. Bachhawat Life Time Achievement Award of Indian Academy of Neurosciences (2018). He is currently the President of the Indian Academy of Neurosciences. He has made significant contribution to glial neurobiology with special reference to the role of microglia and astrocytes in neurodegeneration and the perpetuating effect of glia on the health of the neurons and vice versa. He was the Coordinator of the DBT National Initiative on Glial Cell Research in Health and Disease. He has published more than 90 research papers and has guided 6 M.Phil. and 29 Ph.D. theses. His outstanding contribution to Human Resource Development in Neuroscience (M.Sc. and Ph.D. in Neuroscience since 2001) by establishment of India's 1st UTD of Neuroscience at Jiwaji University, Gwalior, deserves a special mention.

Pankaj Seth is working as a Senior Professor at the National Brain Research Centre, Manesar, an Institute of Excellence in India. He obtained his Ph.D. in Medical Biochemistry from the University of Kanpur, and his Post-Doctoral training at the National Institute of Disorders and Stroke, the National Institutes of Health, Bethesda, USA. He has 25 years of research and 18 years of teaching experience. He is an elected Fellow of the National Academy of Sciences (India), the National Academy of Medical Sciences, and the Indian Academy of Neurosciences. Dr. Seth has made immense contributions in glial biology, particularly in understanding the role of astrocytes in viral neuropathogenesis. He has published more than 70 research papers, 8 book chapters and editorials. He is recipient of several national and international awards, serves on editorial boards of international journals, and is

council member of the International Society for Neurovirology, USA, and the Asian-Pacific Society for Neurochemistry, Singapore.

Nisha Patro has been working as Research Scientist with support from the Department of Science and Technology and the Department of Biotechnology, Govt. of India. She holds M.Sc. and Ph.D. degrees from Kurukshetra University, Kurukshetra. She had her Post-Doctoral at MRC Neurochemical Pathology Unit, Newcastle upon Tyne, UK. She has 39 years of research and 30 years of teaching experience. She is an elected member of the National Academy of Sciences (India) and elected Fellow of Collegium Internationale Neuropsychopharmalogicum. She has significantly contributed to the areas of development of glia and the role of glia in development of the brain and developmental neurotoxicity. She is recipient of the "Jyotsanamayee Raghunath Bhattacharya Prize" for Best Paper published in 2009–10 by the Indian Academy of Neurosciences. She has completed 5 research projects as a Principal/Co-Principal Investigator and also participated in the DBT National Initiative on Glial Cell Research in Health and Disease. She has 49 research papers and 8 book chapters to her credit.

Prakash Narain Tandon the Doyen of Indian Neuroscience was born on 13.08.1928 at Shimla, India. He received his medical education at K.G. Medical College, Lucknow, (MBBS 1950, First in the University, M.S. 1952), obtained FRCS England in 1956, received training in Neurosurgery at Oslo, Norway and Montreal, Canada. He started the first academic Neurosurgical Unit in Uttar Pradesh in his alma mater (1961) and moved to the All India Institute of Medical Sciences (AIIMS) to establish the first Neurosurgical Department there (1965) which was upgraded as Neurosciences Centre (1975). Following his superannuation, he catalyzed the establishment of the National Brain Research Centre (1997) in which he served as its Founding President (2002).

He has trained more than 50 neurosurgeons who serve all over the country. With the help of various Government agencies (DBT, DST, ICMR, CSIR, and UGC), he promoted overall development of neuroscience.

He had edited 32 monographs, published more than 250 papers, and contributed 50 chapters to various books.

He is one of the most highly recognized medical scientists, being a Fellow of the three Science Academies (President of two of them—INSA, NASI), the National Medical Academy (Vice-President), and the Indian Academy of Neurosciences (President). The Norwegian Academy of Sciences, the Royal Society of Medicine conferred their Fellowship. He was Co-Chair of the IAP (InterAcademy Panel of World Academies of Sciences). He was Honorary Surgeon to the President of India and a Member of the Science Advisory Council of the Prime Minister. Among many of their awards/recognition, he has been awarded the second highest civilian award (Padma Vibhushan) and is currently National Research Professor.

Abbreviations

(11)C-PK11195	1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3 isoquinoline carboxamide
α7nAChRs	Nicotinic acetylcholine receptor of α7 subtype
[11C] DED	[11C]-deuterium-L-deprenyl
18F-FDG	18-Fluorodeoxyglucose
2-AG	2-Arachidonoylglycerol
5-HT	5-Hydroxytryptamine receptors
6-OHDA	6-Hydroxy dopamine
AA	Arachidonic acid
AAV	Adeno-associated virus
AAV	Adeno-associated vector
ACC	Anterior cingulate cortex
AChE	Acetylcholinesterase
ACM	Astrocyte conditioned media
ACT1	NFκB activator 1
AD	Alzheimer's disease
ADEM	Acute disseminated encephalomyelitis
ADHD	Attention deficit hyperactivity disorder
ADK	Adenosine kinase
AEA	N-Arachidonoylethanolamine
AEDs	Antiepileptic drugs
AGE	Aged garlic extract
AGM	Aorta-gonad-mesonephros
AGRP	Agouti-related peptide
AICD	APP intracellular domain
AIDS	Acquired immunodeficiency syndrome
AKT	Protein kinase B
ALDH1	Aldehyde dehydrogenase 1
ALDH1L1	Aldehyde dehydrogenase 1 family member L1
AldoC	Aldolase C
ALK	Anaplastic lymphoma kinase
ALR	AIM2-like receptors
ALS	Amyotrophic lateral sclerosis

ALS-CSF	Cerebrospinal fluid of sporadic amyotrophic lateral sclerosis patients
AlzgI/A-I	Alzheimer's type I
AlzgII/A-II	Alzheimer's type II
AMD	Age-related macular degeneration
AML	Acute myeloid leukemia
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMS	Amyotrophic multiple sclerosis
AMSC	Adipose tissue mesenchymal stem cell
ANLS	Astrocyte-neuron lactate shuttle
ANS	Autonomic nervous system
AP-1	Adaptor protein complex-1/activator protein-1
Apoe	Apolipoprotein E
APP	Amyloid precursor protein
AQP	Aquaporin
AQP4	Aquaporin-4
ARC	Arcuate nucleus
ARE	Antioxidant response element
ASC	Apoptosis-associated speck-like protein containing a caspase recruitment domain
ASD	Autism spectrum disorder
ASH-WEX	Water extract from leaves of *Withania somnifera*
ASK1	Apoptosis signal-regulating kinase
ATP	Adenosine triphosphate
ATP13A2	Polyamine-transporting ATPase 13A2
ATR	Atorvastatin
ATRX	Alpha thalassemia mental retardation, X-linked
Aβ	Amyloid β
BACE1	Beta-site APP cleaving enzyme 1
BBB	Blood–brain barrier
Bcl-xL	B-cell lymphoma-extra large
Bcl2	B-cell lymphoma 2
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BG	Basal ganglia
BLA	Basolateral amygdala
BLBP	Brain lipid-binding protein
BM	Bacopa monnieri
BM	Bone marrow
BM-MSC	Bone marrow-derived mesenchymal stem cell
BMPs	Bone morphogenetic proteins
BMVECs	Brain microvascular endothelial cells
BSCB	Blood–spinal cord barrier
C/EBP	CCAAT enhancer-binding protein
C1q	Complement component 1q

CA	Cornu Ammonis
cAMP	Cyclic adenosine monophosphate
CARD	Caspase activation and recruitment domain
CB	Cerebellum
CB	Cannabinoid receptor
CBA	Cytokine bead array
CBGD	Cortical-basal ganglionic degeneration
CBP	CREB-binding proteins
CCCP	Carbonyl cyanide m-chlorophenyl hydrazone
CCL-3	Chemokine (C-C motif) ligand 3
CCL-2	C-C motif chemokine ligand 2
CD	Cluster of differentiation
Cdk5	Cyclin-dependent kinases 5
CDKN2A/2B	Cyclin-dependent kinase inhibitor 2A/2B
CFU-F	Colonyforming unit formation
CHIKV	Chikungunya virus
CHIT-1	Chitotriosidase-1
chR2	Channelrhodopsin-2 receptor
cIMPACT-	The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy
CINC	Cytokine-induced neutrophil chemoattractant
CJD	Creutzfeldt-Jakob disease
CLN	Claudin
ClpB	Caseinolytic peptidase B
CLR	C-type lectin receptors
Clu	Clusterin
CM	Cerebral malaria
CMA	Chaperone-mediated autophagy
CMT	Charcot-Marie-Tooth disease
CMV	Cytomegalovirus
CN	Caudate nucleus
CNP	Cyclic nucleotide phosphodiesterase
CNPase	2', 3'-Cyclic nucleotide 3'-phosphodiesterase
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
COX	Cyclooxygenase
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CREB	Cyclic-AMP response element-binding protein
CRP	C-reactive protein
CRYAB	Crystallin alpha B ER-α, estrogen receptor alpha
CSDS	Chronic social defeat stress
CSF	Cerebrospinal fluid
CSF-1	Colony-stimulating factor-1

CSF-1R	Colony-stimulating factor-1 receptor
CSPGs	Chondroitin sulfate proteoglycans
CT-1	Cardiotrophin-1
CUMS	Chronic unpredictable mild stress
CUS	Chronic unpredictable stress
Cx	Connexin
CX-30	Connexin-30
CX-43	Connexin-43
CX3C1	C-X3-C Chemokine fractalkine
CX3CR1	C-X3-C Chemokine receptor
CXCL	C-X-C Motif chemokine ligand
CXCL12	C-X-C Motif chemokine ligand 12
CXCR-4	C-X-C Chemokine receptor type 4
CXs	Connexins
DA	Dopaminergic
DAA	Disease-associated astrocytes
DAG	1, 2-Diacylglycerol
DAGL	DAG lipase
DAM	Disease-associated microglia
DAMPs	Damage-associated molecular patterns
DAP12	DNAX activation protein 12
DAT	Dopamine amino transporter
DBS	Deep brain stimulation therapy
DCFDA	Dichloro-dihydrofluorescein diacetate
DDR1	Dopamine D1 receptor
DENV	Dengue virus
dHMN	Distal hereditary motor neuropathy
DJ-1	Deglycase
DLB	Dementia with Lewy body disease
dlPFC	Dorsolateral prefrontal cortex
DMPFC	Dorsomedial prefrontal cortex
DMT	Divalent metal transporter
DMT1	Divalent metal transporter 1
DNA	Deoxyribonucleic acid
Dnmt1	DNA methylating enzyme 1
DPI	Diphenyleneiodonium chloride
DPSC	Dental pulp stem cell
DR	Diabetic retinopathy
DRD1	Dopamine receptor 1
DRD2	Dopamine receptor 2
DRE	Drug-resistant epilepsy
DREADD	Designer receptors exclusively activated by designer drugs
DRG	Dorsal root ganglia
DWm	Mitochondrial membrane potential
E-Protein	Envelope protein

EAAT-1	Excitatory amino acid transporter-1
EAAT-2	Excitatory amino acid transporter-2
EAE	Experimental autoimmune encephalitis
eCB	Endocannabinoids
ECE-2	Endothelin-converting enzyme-2
ECM	Extracellular matrix
ECS	Endocannabinoid system
ECS	Extracellular space
ECT	Electroconvulsive therapy
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EMPs	Erythromyeloid progenitors
eNOS	Endothelial nitric oxide synthase
ENT	Equilibrative nucleoside transporter
EP2	Prostaglandin E2
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
ERK1/2	Extracellular signal-regulated protein kinase ½
ESC	Embryonic stem cell
Ets	E-twenty-six
FAAH	Fatty acid amide hydrolase
FABP7	Fatty acid-binding protein
FALS	Familial amyotrophic lateral sclerosis
FBS	Fetal bovine serum
FC	Frontal cortex
FCD	Focal cortical dysplasia
FCRLs	Fc receptor-like molecules
FDG-PET	Fluoro-2-deoxy-D-glucose positron emission tomography
FFPE	Formalin-fixed paraffin embedded
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FL	Fetal liver
FLP-FRT	Flippase-flippase recognition target
fMRI	Functional MRI
FS	Febrile seizures
FUS/TLS	Fused in sarcoma/translocated in liposarcoma
G-CSF	Granulocyte colony-stimulating factor
G6PDH	Glucose-6-phosphate dehydrogenase
GABA	Gamma-aminobutyric acid
GABA-T	GABA transaminase
GAD	Glutamate decarboxylase
GAD67	Glutamic acid decarboxylase
GAL9	Galectin-9

GALC	Galactocerebroside
GAP-43	Growth-associated protein-43
GAT	GABA transporters
GB	Glioblast
GB	Glioblastoma
GBA	Glucocerebrosidase
GCI	Glial cell/cytoplasmic inclusions
GCM	Glial conditioned medium
Gcm	Glial cells missing
GCM-LGF	Media of LGF-treated glial cultures
GD	Gestation Day
GDNF	Glial cell line-derived neurotrophic factor
GE	Glucose excited
GFAP	Glial fibrillary acidic protein
GFAP-ir cells	Glia fibrillary acidic protein-immunoreactive cells
GGF2	Glial growth factor 2
Ggta1	Glycoprotein alpha-galactosyltransferase 1
GHSR	Growth hormone secretagogue receptor
GI	Glucose inhibited
GJs	Gap junctions
GK	Glucokinase
GLAST	Glutamine aspartate transporter
GLE	Extracts of Ganoderma lucidum
GlnC4	Glutamine-C4
GLP	Polysaccharide of Ganoderma lucidum
GLP1R	Glucagon-like peptide-1 receptor
GLT	Glutamate transporter 1
Glu R	Glutamate receptor rich
GluC4	Glutamate-C4
GLUT	Glucose transporters
Glx	Glutamine-glutamate
GM-CSF	Granulocyte macrophage colony-stimulating factor
GMC	Ganglion mother cell
GP	Globus pallidus
gp130	Glycoprotein 130
Gp41	Glycoprotein 41
GPC	Glial precursor cells
Gpc4,6	Glypican 4 and 6
GPCRs	G-protein-coupled receptors
GPe	Globus pallidus externa
GPi	Globus pallidus interna
Gpr34	G-protein-coupled receptor 34
GPx	Glutathione
GR	Glucocorticoid receptor
Grp78	Glucose-related protein-78 kDa

GRPs	Glial-restricted progenitors
GS	Glutamine synthetase
GSK3	Glycogen synthase kinase 3
GWAS	Genome-wide association study
H2-D1	Histocompatibility 2, D region locus 1
H_2O_2	Hydrogen peroxide
H4Ac	Acetylation of histone H4
HBV	Hepatitis B virus
HD	Huntington's disease
HDLS	Hereditary diffuse leukoencephalopathy with spheroids
HES	Hairy and enhancer of split-1
hES-AS	Human embryonic stem cell-derived astrocytes
hESC	Human embryonic stem cell
HfNSCs	Human fetal neural stem cells
HHV6	Human herpes virus 6
HIF1	Hypoxia-inducible factor 1
hiPSC	Human-induced pluripotent stem cell
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HLA-DR	Human leukocyte antigen-DR isotype
hM3Dq	Modified human M3 muscarinic (hM3) receptor
HMGB1	High mobility group box 1
HNPCs	Human neural progenitor cells
HNSCC	Head and neck squamous cell carcinoma
HO	Heme oxygenase
HO-1	Heme oxygenase 1
Hox	Homeobox gene
HPA	Hypothalamus pituitary adrenal
HPC	Hippocampus
hPSC	Human pluripotent stem cell
HS	Hippocampal sclerosis
Hsc-70	Heat shock cognate 71 kDa protein
HSCs	Hematopoietic stem cells
HSP	Heat shock protein
HSV	Herpes simplex virus
Iba 1	Ionized calcium-binding adaptor molecule 1
ICAM	Intercellular adhesion molecule
Id2,4	Inhibitors of differentiation 2,4
IDE	Insulin-degrading enzyme
IDH	Isocitrate dehydrogenase
IDO	Indoleamine 2, 3-dioxygenase 1
IF	Immunofluorescence
IFN	Interferon
IFN-γ	Interferon-γ
IGF	Insulin-like growth factor

IGF-1	Insulin-like growth factor-1
IHC	Immunohistochemistry
IL	Interleukin
IL- 8, 12	Interleukin-8, 12
IL-1r	Interleukin-1 receptor
IL-1ra	Interleukin-1 receptor antagonist
IL-1RAcP	Interleukin-1 receptor accessory protein
IL-1β	Interleukin 1β
IL-34	Interleukin-34
IL-4, 5	Interleukin-4, 5
IL-6	Interleukin-6
ILAE	International League Against Epilepsy
iNOS	Inducible nitric oxide synthase
IOP	Intraocular pressure
iPLA2β	Phospholipase A2 beta
iPSC	Induced pluripotent cells
IRAK	Interleukin-1 receptor-associated kinase
IRF	Interferon regulatory factor
IRF8	Interferon regulatory factor 8
ISGs	Interferon-stimulated genes
ISRE	Interferon-stimulated response element
IT-type	Intratelencephalic connections
Itgax	Integrin subunit alpha X
JAK	Janus activated kinase
JAK/STAT	Janus kinase-signal transducer and activator of transcription
JCV	JC virus
JEV	Japanese encephalitis virus
JNK	c-JUN N-terminal kinase
KATs	Kynurenine aminotransferases
KCC2	K-Cl co-transporter
kDa	Kilodalton
Keap	Kelch-like ECH-associated protein
Kir4.1	Inwardly rectifying K^+ channel 4.1
Klf4	Krüppel-like factor
KMO	Kynurenine-3-monooxygenase
KO	Knockout
KSR	Kinase suppressor of Ras
KYN	Kynurenine
L-DOPA	Levodopa
L-NAME	NG-nitro-L-arginine methyl ester
L-α-AA	L-α-aminoadipate
LACV	La Crosse virus
LAMP	Lysosomal-associated membrane protein
LAMP2	Lysosomal-associated membrane protein 2
LB	Lewy body

LC	Locus coeruleus
LC3	Microtubule-associated protein light chain 3
LCM	Laser capture microdissection
LDH	Lactate dehydrogenase
LDH5	Lactate dehydrogenase 5
LepR	Leptin receptor
LGF	Liver growth factor
LIF	Leukemia inhibitory factor
LIFRβ	Leukemia inhibitory factor receptor β
LIP	Labile iron pool
lncRNAs	Long non-coding RNAs
LOFC	Left orbitofrontal cortex
LOX	Lipoxygenase
LPS	Lipopolysaccharide
LRP1	Lipoprotein receptor-related protein 1
LRR	Leucine-rich repeat
LRRK2	Leucine-rich repeat kinase 2
LTA	Lipoteichoic acid
LTD	Long-term depression
LTP	Long-term potentiation
MAFB	MAF BZIP transcription factor B
MAG	Myelin-associated glycoprotein
MAGL	Monoacylglycerol lipase
MALDI/TOF	Matrix-assisted laser desorption/ionization time-of-flight
MANF	Mesencephalic astrocyte-derived neurotrophic factor
MAO	Monoamine oxidase
MAO-B	Monoamine oxidase B
MAP-2	Microtubule-associated protein
MAPK	Mitogen-activated protein kinase
MARCM	Mosaic analysis with a repressible cellular marker
MAVS	Mitochondrial antiviral-signaling protein
MBP	Myelin basic protein
MC	Motor cortex
MCAO	Middle cerebral artery occlusion
MCP-1	Monocyte chemoattractant protein-1
MCT	Monocarboxylate transporters
MDD	Major depressive disorder
MDT	Mediodorsal thalamus
MECP2	Methyl-CpG-binding protein 2
MEGF10	Multiple epidermal growth factor-like domains protein 10
MEIS3	Meis homeobox 3
MEK	Mitogen-activated protein kinase ERK kinase
MERTK	MER proto-oncogene tyrosine kinase
METH	Methamphetamine
mGluR1	Metabotropic glutamate receptor 1

mGluRs	Metabotropic glutamate receptors
MGMT	Promoter methylation of $O6^6$-methylguanine-DNA methyl-transferase
MGnD	Microglial neurodegenerative phenotype
MHC	Major histocompatibility complex
MHCII	Major histocompatibility complex II
mHTT	Mutant Huntingtin
MIF	Macrophage inhibitory factor
MIP-1α	Macrophage inflammatory protein-1 α
miRNAs	MicroRNAs
MJD	Machado-Joseph disease
MLD	Metachromatic leukodystrophy ()
MMP-3	Metalloproteinase-3
MMP-9	Matrix metalloproteinase-9
MMPs	Matrix metalloproteinases
MNNG	N-Methyl-N'-nitro-N-nitrosoguanidine PARP
MOBP	Myelin oligodendrocyte basic protein
MOG	Myelin oligodendrocyte protein
mPFC	Medial prefrontal cortex
MPP+	1-Methyl-4-phenylpyridinium
MPS	Mononuclear phagocyte system
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance imaging
MRP	Multidrug resistance protein
MRP1	Multidrug resistance protein 1
MRS	Magnetic resonance spectroscopy
MS	Mass spectrometry
MS	Multiple sclerosis
MSA	Multiple system atrophy
MSC	Mesenchymal stem cell
MSNs	Medium spiny neurons
mSOD-1	Mutant superoxide dismutase-1
MTS	Mesial temporal sclerosis
MV	Microvesicle
Myb	Transcription factor myeloblastosis
MyD88	Myeloid differentiation primary response 88
MyRF	Myelin gene regulatory factor
NAA	N-Acetyl aspartate
NAD+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide + hydrogen (H)
NALS-CSF	CSF of non-ALS patients
NAPE-PLD	Phosphatidylethanolamine phospholipase D
NB	Neuroblast
NC	Normal control
NCC	Neurocysticercosis

NCX	Na^+/Ca^{2+} exchanger
NDRG2	N-myc downregulated gene 2
NEP	Neprilysin
NEPs	Neural epithelial precursors
NeuroD	Neuronal differentiation gene
NF	Neurotrophic factor
NF-H	Neurofilament-H
NF-κB	Nuclear factor kappa B
NF1A	Nuclear factor 1A
NFATp	Nuclear factor of activated T cells p
NFTs	Neurofibrillary tangles
NG2	Nerve/glial antigen 2
NGB	Neuroglioblast
NGF	Nerve growth factor
Ngn1	*Neurogenin1*
NHD	Nasu-Hakola disease
NKCC1	Na-K-Cl co-transporter
NKRF	NF-κB repressing factor
NL	Neuroligin
NLR	Nucleotide-binding oligomerization domain-like receptor
NLRP3	NACHT, LRR, and PYD domains-containing protein-3
NLRs	NOD-like receptors
NMDA	N-Methyl D-aspartate
NMO	Neuromyelitis optica
NMOSD	Neuromyelitis optica spectrum disorder
NMR	Nuclear magnetic resonance
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
NOS	Nitrogen oxidative species
NOX	NADPH-oxidized
Nox2	NADPH oxidase 2
NPC	Neural progenitor cells
NPY	Neuropeptide Y
NQO	NAD(P)H quinone dehydrogenase
Nrf-2	Nuclear factor erythroid 2-related factor 2
Nrf2-ARE	Nuclear factor erythroid 2-related factor 2–antioxidant response element
NSAIDs	Nonsteroidal anti-inflammatory drugs
NSC-34	Neuroblastoma X spinal cord hybrid cell line
NSCs	Neural stem cells
NT3	Neurotrophin-3
NVU	Neurovascular unit
OCD	Obsessive compulsive disorder
OCLN	Occludin

ODG	Oligodendroglioma
OFC	Orbitofrontal cortex
OL	Oligodendrocyte
Olfm13	Olfactomedin-like 3
OMT	Oxymatrine
ONOO-	Peroxynitrite
OPCA	Olivopontocerebellar atrophy
OPCs	Oligodendrocyte precursor cells
P	Postnatal
P2X7R	P2X purinoceptor 7
P2Y11	P2Y purinoceptor 11
P2Y12R	Purinergic receptor 12
PA	Pilocytic astrocytoma
PAG	Phosphate-activated glutaminase
PAM	Plaque-associated microglia
PAMP	Pathogen-associated molecular patterns
PARK-7	Parkinson disease protein 7
PARP	Poly(ADP-ribose) polymerase
Pax	Paired box gene
PB2	Polymerase basic protein-2
PBMCs	Peripheral blood mononuclear cells
PBR	Peripheral benzodiazepine receptor
PC	Prelimbic cortex
PCD	Programmed cell death
PD	Parkinson's disease
PDA	Progenitor-derived astrocytes
PDGF	Platelet-derived growth factor
PDGFRα	Platelet-derived growth factor receptor α
PET	Positron emission tomography
PFC	Prefrontal cortex
PFK	Phosphofructokinase
PGE-2	Prostaglandin E2
PHGG	Pediatric high-grade gliomas
PI3K	Phosphoinositide 3-kinases
PINK1	PTEN-induced putative kinase-1
PK	Pyruvate kinase
PK-KO	Parkin knockout
PKC	Protein kinase C
PLA2G6	Phospholipase A2 group VI
PLC	Pontine locus coeruleus
PLC	Phospholipase-C
PLD	Phospholipase-D
PLGG	Pediatric low-grade gliomas
PLP	Proteolipid protein 1
PMD	Pelizaeus-Merzbacher disease

PML	Progressive multifocal leukoencephalopathy
PMP22	Peripheral myelin protein
PNNSL	Perineural nets
PNS	Peripheral nervous system
Poly(Q)	Polyglutamine
POMS	Pro-opiomelanocortin
PPARs	Peroxisome proliferator-activated receptors
PRR	Pattern recognition receptor
PSD-95	Post-synaptic marker protein
PSEN	Presenilin
PSP	Progressive supranuclear palsy
PT-type	Pyramidal tract
PTFE	Polytetrafluoroethylene
PTPRC	Protein tyrosine phosphatase receptor type C
PTX3	Pentraxin-3
PTZ	Pentylenetetrazole
PUFA	Polyunsaturated fatty acids
PXA	Pleomorphic xanthoastrocytoma
PYD	Pyrin domain
QUIN	Quinolinic acid
RAGE	Receptor for advanced glycation end products
RANTES	Regulated upon activation, normal T-cell expressed and presumably secreted
RAS	Retrovirus-associated DNA sequences
RG	Radial glia
RIG-1	Retinoic acid-inducible gene-1
RLRs	RIG-I-like receptors
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RP	Retinitis pigmentosa
RSCs	Remak Schwann cells
RU486	Roussel-Uclaf 486
Runx1	Runt-related transcription factor
S100a10	S100 calcium-binding protein a10
S100β	S100 calcium-binding protein β
SAC	S-Allyl cysteine
SALL1 & 3	Spalt-like transcription factors 1&3
SALS	Sporadic amyotrophic lateral sclerosis
SAP-C1q	Serum amyloid P-complement component 1, q subcomponent
SASP	Senescence-associated secretory phenotype
SC	Schwann cells
SCA1	Spinocerebellar ataxia type 1
SCI	Spinal cord injury
SDS	Shy-Drager syndrome
SEGA	Subependymal giant cell astrocytoma

SGC	Satellite glial cells
SHH	Sonic hedgehog
sICAM-1	Soluble intercellular adhesion molecule 1
SICs	Slow inward currents
SiglecH	Sialic acid-binding immunoglobulin-like lectin H
Sip1	Smad-interacting protein 1
SLC1A2	Solute carrier family 1 member 2
SLC1A3	Solute carrier family 1 member 3
Smarca-4/Brg1	ATP-dependent SWI/SNF chromatin remodeling enzyme
SNATs	Sodium-coupled neutral amino acid transporter
SNC	Sciatic nerve crush
SNCA	α-synuclein gene
SND	Striato-nigral degeneration
SNpc	Substantia nigra pars compacta
SNpr	Substantia nigra pars reticulata
SNV	Single nucleotide variation
SOCS3	Suppressor of cytokine signaling 3
SOD-1	Superoxide dismutase-1
SOD-2	Superoxide dismutase-2
SPARC	Secreted protein acidic and cysteine rich
SPG2	Severe spastic paraplegia 2
SPHK1	Sphingosine kinase 1
Spp1	Secreted phosphoprotein 1
SRB1	Scavenger receptor class B member 1
SREBP	Sterol regulatory element-binding protein
SSADH	Succinic semi-aldehyde dehydrogenase
SSP1	Sporulation-specific protein 1
SSPE	Subacute-sclerosing panencephalitis
SSRIs	Selective serotonin reuptake inhibitors
STAT3	Signal transducer and activator of transcription 3
STN	Subthalamic nucleus
SVZ	Subventricular zone
T3	Triiodothyronine
T4	Thyroxine
Tat	Transactivator of transcription
TBEV	Tick-borne encephalitis virus
tBHQ	Tertiary butylhydroquinone
TBI	Traumatic brain injury
TBM	Tubercular meningitis
TCA	Tricarboxylic acid cycle
TCE	50% ethanolic extract of *Tinospora cordifolia*
TDP-43	43-kDa transactive response (TAR)-DNA-binding protein
TEER	Transendothelial electrical resistance
TERT	Telomerase reverse transcriptase
Tf	Transferrin

Tfam	Mitochondrial transcription factor A
TFR	Transferrin receptor
TG	Trigeminal ganglia
TGF-α	Transforming growth factor-α
TGF-β	Transforming growth factor beta
TH	Tyrosine hydroxylase
TH-ir	Tyrosine hydroxylase-immunoreactive
Thbs1	Thrombospondin 1
TIMP-1	Tissue inhibitor of matrix metalloproteinase 1
TIMPs	Tissue inhibitors of metalloproteinases
TLE	Temporal lobe epilepsy
TLR	Toll-like receptor
TLR4	Toll-like receptor 4
TM4SF1	Transmembrane 4 L six family member 1
TMEM119	Transmembrane protein 119
TMEV	Theiler's murine encephalomyelitis virus
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor alpha
TNFR1	TNF receptor type 1
TNFR2	TNF receptor type 2
TRAF-6	Tumor necrosis factor receptor-associated factor 6
TRAP-1	TNF receptor-associated protein
TREM-2	Triggering receptor expressed on myeloid cells 2
TRIF	TIR-domain-containing adaptor-inducing interferon-β
TrkB	Tropomyosin receptor kinase B
TRPC3	Transient receptor potential cation channel subfamily C member 3
TRPV1	Transient receptor potential vanilloid type-1
TSH	Thyroid-stimulating hormone
TSPO	Translocator protein
VC	Visual cortex
VCAM	Vascular cell adhesion molecule
VDAC1	Voltage-dependent anion-selective channel 1
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor A
VEGFR-1	Vascular endothelial growth factor receptor-1
VGSC	Voltage-gated sodium channel
VIM	Vimentin
VM DA	Ventral midbrain dopaminergic
VMN	Ventromedial nuclei
vmPFC	Ventromedial prefrontal cortex
VNUT	Vesicular nucleotide transporter
VPA	Valproic acid
VPFC	Ventral prefrontal cortex
VTA	Ventral tegmental area

VWM	Vanishing white matter disease
VZ	Ventricular zone
WD	Wilson's disease
WHO	World Health Organization
WM	White matter
Wnt	Wingless/integrated
WNV	West Nile Virus
WT	Wild type
YS	Yolk sac
Zfp24	Zinc finger protein 24
ZIKV	Zika virus
ZO	Zonula occludens
ZO-1	Zonula occludens-1
μPIXE	Scanning proton-induced X-ray emission

Glial Biology: A Historical Perspective

P. N. Tandon

Abstract

In spite of the fact that the glial cells were discovered as "neuroglia" as far back as 1854 they remained to be further designated as astrocytes, oligodendrocytes, and microglia up to 1924. Unlike neurons, the glial cells did not get serious attention for almost 100 years and these cells remained to be described as "glue," "servants of the neurons," and so on until the later part of nineteenth and early twentieth century. They gained importance only in the 1980s when neuroscientists realized the interplay between the neurons and glia particularly with the development of modern technologies in understanding cell-cell interactions. Now we understand that the formation, maturation, functioning, and maintenance of neurons are essentially dependent on the glial cells. This chapter intends to summarize the historical perspectives of these developments.

Keywords

Astrocyte · Oligodendrocyte · Microglia · Neuroinflammation

1 Introduction

First described by Virchow in 1854 (neuroglia), further defined by Cajal in 1897 (astrocytes), and better delineated by Hortega in 1919 (microglia and oligodendrocytes), the glial cells in the central nervous system were relegated to be the supporters and "servants" of the neurons, the "regal" constituents of the CNS, for more than a century. Slowly their other characteristics and functions came to be

P. N. Tandon (✉)
National Brain Research Centre, Manesar, Haryana, India
e-mail: tandon@nbrc.ac.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_1

recognized. While during the latter part of the nineteenth and the early twentieth century pathbreaking advances were made in respect to the neuroanatomical, neurophysiological, and neurochemical features of the neurons by His (1831–1904), Meynert (1833–1892), Kraepelin (1856–1926), Sherrington (1857–1952), Langley (1852–1925), Brodmann (1868–1918), Dale (1875–1968), and Adrian (1889–1977) among many others, glial biology did not receive similar attention. Studies on neuroglia had to wait till the 1980s when cellular biology and neurosciences underwent a revolution led by the development of molecular biology, genomics, and genetic engineering and a host of new technologies. Today, one can study glial biology in vivo in real life.

1.1 Astrocytes

The morphological heterogeneity of astrocytes, their orderly arrangement covering specific territory, their participation in "tripartite" "synapses," and their control of cerebral vasculature have now been established. They are excitable in a manner different from neurons. Details of astrocyte-neuron cross-talk and their control of synaptic transmission have been elaborated. They express a wide variety of functional neurotransmitter receptors and release a variety of gliotransmitters. They not only regulate cerebral circulation in response to neuronal activity but also play an active role in brain energy production, delivery, utilization, and storage. They play an important role in brain development, neuronal differentiation, and neuritic outgrowth. Astrocytes not only regulate neurogenesis but are also the neuronal progenitors. They integrate and process synaptic information and finally regulate synaptic transmission and plasticity.

Astrocytes secrete a variety of membrane-associated molecules and participate in neuroinflammation and several other brain pathologies.

1.2 Oligodendrocytes

First clearly defined as a distinct entity by Río Hortega and Wilder Penfield in the 1920s (Penfield 1924), oligodendrocytes have been a subject of detailed studies in recent years in respect to their origin and development. They are all derived from oligodendrocyte progenitor cells (OPCs) and cannot multiply on their own. Axonal activity normally controls the production and/or release of the growth factors that are responsible for the proliferation of OPCs and hence the oligodendrocyte. Their primary function is myelination of the axons in the CNS.

It is now well established that there is a cross-talk between the neurons and oligodendrocytes for the initiation of myelination. Myelination itself is a complex process which has recently been elaborated to a great extent.

It has been shown that oligodendroglia not only myelinate the axons but also provide trophic support to neurons by the production of neurotrophic factors like GDNF, BDNF, and IGF1.

In addition to myelination, oligodendrocytes and OPCs have immunomodulatory function. Besides MS, oligodendroglia have been found to be involved in a host of other CNS pathologies like ischemia, stroke, injuries, inflammation, and infection. They are easily destroyed by antibodies in diseases like neuromyelitis optica (Devic's disease) and autoimmune encephalomyelitis. They are found to be affected in patients of Alzheimer's disease, schizophrenia, and amyotrophic lateral sclerosis.

1.3 Microglia

They were first described as "rod cell" in histopathological studies on the brain of dementia patients by Franz Nissl in 1880. Their morphological details were illustrated by Del Río-Hortega (1919, 1920, 1932), and they were designated as "microglia" to distinguish them from astrocytes and neurons. They are of mesodermal and not of ectodermal origin.

Microglia are the immunocompetent residual macrophages of the CNS responsible for innate immunity. They kill invading microorganisms in the brain, remove debris, and facilitate tissue repair after injury. When activated, microglia release a number of immunocompetent molecules and chemokines which control neuroinflammation and regulate immune response of the brain.

While the role of microglia in acute insult to the brain was well known, it is only recently that their role in neuroinflammation associated with neurodegeneration has been brought to light.

2 Biology of Glia: Part I—Astrocytes

2.1 History

The study of human glia dates back to 1854 when Virchow (1821–1902) described the cellular nature of the cerebral interstitial substance which he called "Nervenkitt." Two years later, he named it "neuroglia" (Greek word for glue) (Somjen 1988). He believed the neuroglia to be of connective tissue origin, functioning as glue between the nerve cells (Oberheim et al. 2009). Using black chrome silver reaction, Camillo Golgi (1843–1926) in 1885 (Golgi 1885) was able to illustrate these better, thus confirming these to be undoubtedly different from neurons. He later termed these as radial glia or multipolar glia. Golgi pointed out that in the brain the glia cells are interposed between blood vessels and neurons and assumed that they convey nutritive substance to the neurons. Soon after, Andriezen published a paper, "The neuroglial elements in human brain" (BMJ.2 (1700) 227–230, 1893) providing further details. A year later, Retzius using Golgi stain illustrated the morphological diversity of glial cells in the human cortex. Ramón y Cajal (1852–1934), who primarily concentrated on neurons, published two pioneering papers in 1897 and 1913 mostly dealing with the function of the glia, "Something about the physiological significance of neuroglia" (Cajal 1897) and "A contribution to the understanding

of neuroglia in the human brain" (Cajal 1913) [Quoted by Navarrete and Araque 2014]. "Algo Sobre La signification functional de la neuroglia" (1897) and "Contributional conocimante de la neuroglia del cerebro—humano" (1913). It is to his credit that Cajal along with a number of his students illustrated the relationship of glial cells, mostly astrocytes, to the neurons, synapses, and blood vessels (Newman 2017). His studies thus provided the concept of "tripartite synapse," "neurovascular coupling," and "astrocyte-neuron interaction."

Andriezen (1893) and Ritzius (1894) provided valuable information about the morphology of the astrocytes. However, Albert von Kolliker (1817–1905) has been credited to distinguish two types of astrocytes named Kurzstrahler later called protoplasmic astrocytes and Langstrahper, i.e., the fibrous astrocytes (Von Kolliker 1919) (Quoted by Sierra et al. 2016). According to Kettenmann and Verkhratsky (2008) and Matyash and Kettenmann (2010), it was von Lenhossek (1863–1937) who coined the term "astrocytes" in 1895 (Lenhossek 1895) in the second edition of his textbook on the nervous system. He classified astrocytes as protoplasmic and fibrous.

Around the same time, Franz Nissl (1860–1918) was the first to recognize microglia as a distinct entity but named them "Stabehenzellen" (rod cells) in 1899. However, it was Pio del Río Hortega (1882–1945), along with his pupil Collado, who using silver sodium carbonate stain first described the various forms of microglia—globose and amoeboid during development and actively phagocyte forms and resting mature form in adults (Penfield 1928; Barron 1995).

Working in Hortega's laboratory in Spain, Wilder Penfield (1891–1976) was able to stain the oligodendrocytes for the first time. This was published in 1924 (Penfield 1924) in *Brain* giving a detailed account of these cells. A recent paper by Kuhn et al. (2019) provides a detailed account of these cells.

While during the latter part of the nineteenth and early part of the twentieth century path-breaking advances were made in respect to neuroanatomical, neurophysiological, and neurochemical studies of the neurons, mostly in the UK and Europe, little attention was paid to neuroglia. These pioneer neuroscientists included Franz Nissl (1860–1918), Wilhelm His (1831–1904), Theodor Meynert (1833–1892), Emil Kraepelin (1856–1926), Gustav Retzius (1842–1919), Charles Sherrington (1857–1952), von Economo (1876–1931), John Langley (1851–1925), Korbinian Brodmann (1868–1918), Henry Dale (1875–1968), and Edgar Adrian (1889–1977).

According to Khakh and Sofroniew (2015), Kuffler (1967) was the first to predict the role of neuroglia in neuronal activity which initiated the emerging field of glial biology. Thus, detailed studies on neuroglia had to wait till 1980 when cellular biology and neurosciences underwent a revolution by the developments in molecular biology, genomics, and genetic engineering. This was supported by new technologies and the development of new tools such as the patch-clamp technique, fluorescence imaging, and confocal and multiphoton microscopy which allowed the detailed visualization of the structural and physiological processes of the cells. The outcomes of these investigations, some of which permit in vivo visualization of their activities, are described in respect to individual glial cell categories. It must,

however, be pointed out these cells do not function individually independently but in collaboration with each other and the neurons for a particular goal.

In short, there have been recent advances in respect to the structure, distribution, organization, and functions of astrocytes hitherto unknown (Volterra and Meldolesi 2005). Verkhratsky and Nedergaard (2018) have provided a very detailed account of "physiology of astroglia." This chapter provides an update on the current knowledge on astrocytes.

Astrocytes are the largest number of cells in the CNS. Though already recognized as a distinct entity, it took more than a century to unveil their true functions, not just as a "glue" but as essential participants in the development and functions of the neurons themselves. Interestingly, *von Lenhossek* who in 1895 coined the word "astrocytes" recognized these cells to be equivalent to the nerve cells. In short, astrocytes are highly polyvalent cells that are implicated in almost all processes that occur in the CNS such as neurogenesis, synaptogenesis, and bilateral communication with neurons and other glial cells (Yu et al. 2020). The following account highlights some of these features:

2.2 Morphology

Astrocyte's Homogeneity As mentioned earlier, initially, the morphology of astrocytes was described by Andriezen (1893), Retzius (1894), and Cajal (1897), not only in humans but also in other mammals. However, it was Emsley and Macklis (2006) who drew attention to the heterogeneity of astrocytes. They divided astrocytes in nine classes—tanycytes, radial cells, Bergmann glia, protoplasmic astrocytes, fibrous astrocytes, velate glia, marginal glia, perivascular glia, and ependymal glia. Oberheim et al. (2006, 2009) described a novel human-specific subtype of astroglia designated as varicose projection astrocyte. Matyash and Kettenmann (2010) provided a detailed account of the morphology and physiology of astrocytes. They pointed out that the morphology of astrocytes is determined by the cytoarchitecture of a given brain region. Recent studies have confirmed that astrocytic morphology is heterogeneous within and across brain regions and dynamic in both physiological and pathological states (Yu et al. 2020; Khakh and Sofroniew 2015; Khakh and Deneen 2019).

Astrocytes manifest heterogeneity of membrane currents, glutamate receptor expression, expression of other transmitter receptors, gap junction coupling, and Ca2+ signaling (Matayash and Kettenmann 2010).

Instead of being considered to be distributed haphazardly, it is now known that they exist in an orderly arrangement with minimal overlap. They cover specific territory that interfaces with the microvasculature that might include thousands of synapses. The fraction of this territory can be controlled by specialized astrocyte micro domains which allow highly dynamic interaction with surrounding synapses (Bushong et al. 2002; Oberheim et al. 2006; Khakh and Sofroniew 2015; Khakh and Deneen 2019).

Morphologically and most likely functionally human astrocytes differ from those of rodents. The human cortical astrocytes are larger and structurally more complex and more diverse than those of the rodents (Oberheim et al. 2009). One of the most distinguishing features of the adult human brain is the complexity and diversity of its cortical astrocytes. In all mammals, protoplasmic astrocytes are organized into a spatially non-overlapping domain that encompasses both neurons and vasculature. However, unique to both humans and primates are additional populations of interlaminar astrocytes that also project distinctive long process, frequently un-branched, throughout the layers of the cortex, terminating in either layer 3 or 4. They were already described by Andriezen and Retzius in 1890 (Oberheim et al. 2006).

The most important marker of astrocytes is glial fibrillary acidic protein (GFAP) which is found in almost all reactive astrocytes during central nervous system injury. Other putative markers for astrocytes are S100B. Barres (2008) has provided a list of other markers used for identification of astrocytes.

2.3 Functions

It is now well established that astrocytes have key role in brain development and functions such as neuronal metabolism, synaptogenesis, homeostasis of the extracellular milieu, and cerebral microcirculation. The biology of astrocyte-neuron interaction has emerged as a rapidly expanding field in the 1990s and has become the most exciting topic in current physiology that is changing our vision of the physiology of the nervous system (Perea et al. 2008; Verkhratsky and Nedergaard 2018). This paper by Alexei Verkhratsky and Nedergaard (2018) is an extensive review on the physiology of astroglia in *Physiology Reviews* 98, 239–389. Astrocytes are highly polyvalent cells that are implicated in almost all processes of CNS functioning including local integration, synaptic and non-synaptic communication, neurogenesis, and synaptogenesis (Yu et al. 2020).

Astrocyte Excitability

Till recently considered to be non-excitable support cells of the brain, it is now established that astrocytes are excitable, based on their distinct physiology quite different from neurons. Numerous studies performed during the past few years have established a form of cellular excitability of astrocytes based on variation of Ca2+ concentration in cytosol rather than electrical changes in the cell membrane, a characteristic of neurons. This excitability is regulated by Ca2+ levels in the cells. In the late 1980s, astrocytes were found to express voltage-gated channels and neurotransmitter receptors (Volterra and Meldosi 2005; Barres 2008; Matyash and Kettenmann 2010).

Studies performed in cultured cells, brain slices, and in vivo have firmly established the astrocyte excitability which is manifested as the elevation of cytosolic Ca2+. Fluorescence imaging techniques have shown that Ca2+ elevation occurs spontaneously as intrinsic elements in the absence of neural activity, or they can be triggered by neurotransmitters released during synaptic activity.

Astrocyte Ca2+ elevation has also been observed following physiological sensory stimuli (Perea et al. 2008). Astrocyte Ca2+ elevation stimulates the release of different gliotransmitters. Imaging of astrocytic calcium levels was the initial experimental step of the glial revolution (Haydon 2001).

Astrocyte-Neuron Interaction

Astrocytes express a wide variety of functional neurotransmitter receptors and release several neuroactive molecules such as glutamate, D-serine, ATP, adenosine, GABA, TNFX, prostaglandins, proteins, and peptides. These molecules collectively called as "gliotransmitters" control astrocyte to neuron communication and also synaptic transmission (Perea and Araque 2010; Araque et al. 2014).

It has been demonstrated that signaling between neurons and astrocytes is a reciprocal communication where astrocytes not only respond to neuronal activity but also actively regulate neuronal and synaptic activity (Zonta et al. 2003; Perea and Araque 2006; Haydon and Carmignoto 2006; Newman 2003; Araque and Navarette 2010; Yu et al. 2020).

Astrocytes have an important role in various aspects of brain development and function such as neural metabolism, synaptogenesis, homeostasis of the extracellular milieu, and cerebral circulation. They are involved in neuronal survival and differentiation, neuronal guidance, and neurite outgrowth (Zonta et al. 2003). Interestingly enough studies have produced evidence to suggest that astrocytes not only regulate neurogenesis but are also neural progenitors. It has been shown by anatomical, genetic, and functional studies on humans and other mammals that astrocytes are critical for improved cognitive abilities in humans (Robertson 2014; Zhang and Barres 2013).

There is a yet another function of neuron-astrocyte co-existence in the CNS. They support each other in a variety of ways. Recently, Farmer et al. (2016) explored the influence of neurons on two specialized types of astrocytes in the mouse cerebellar cortex. They found the neurons produced the morphogen sonic Hedgehog. Hedgehog signaling adjusted distinctive gene expression within the two astrocytic cell types. They concluded that mature neurons appear to promote and maintain specific properties of associated astrocytes.

Astrocytes and Synapses

Astrocyte processes envelop the neuronal synapse and give rise to a structure called "tripartite synapse." As mentioned earlier, Cajal (1897) had described this anatomical structure, but it is only recently its functional significance has been elaborated (Gallo and Chittajallu 2001; Perea et al. 2008).

Astrocytes are thus an integral part of the synapse and can be considered as cellular elements involved in synaptic information processing (Perea and Araque 2006, 2010). It is now established that astrocytes sense the activity of neighboring synapses responding to neurotransmitters released by synaptic terminals. Furthermore, astrocytes may in turn influence synaptic transmission (Araque et al. 2001; Haydon 2001; Carmingnoto 2000). Recently, Santello et al. (2019) have explored the role of astrocytes from information processing to cognition and cognitive

impairment. Volterra and Meldolosi (2005) have elaborated how astrocytes "listen and talk." Thus, astrocytes are part and parcel of an integrated network of brain communication, both synaptic and non-synaptic routes. The control of synaptic structure and function depends upon direct neuroglia signaling involving intracellular Ca2+ concentration which is induced by synaptic glutamate-dependent activation of AMPARS (Bezzi and Volterra 2001). Various soluble factors released by astrocytes have been shown to promote formation and maturation of excitatory and inhibitory synapses (Bolton and Eroglu 2009).

The earlier concept of "tripartite synapse" has now been extended to a "multipartite synapse" consisting of (1) the presynaptic terminals; (2) the postsynaptic compartment; (3) the perisynaptic process of neighboring microglial cell that periodically contacts the synaptic structures; and (4) the extracellular matrix (ECM), which is present in the synaptic cleft and extends extrasynaptically.

The role of astroglia in the regulation of synaptic connectivity is, however, immensely wider; astrocytes control emergence and shaping of synaptic network, regulate ionic homeostasis of the synaptic cleft, control neurotransmitter dynamics, present or allow neurotransmitter spillover, and contribute to synaptic extinction (Oliet et al. 2001). These multiple roles of astroglia in synaptic physiology were synthesized in the concept of the "astroglial cradle."

Neuron-dependent excitation of astrocytes is widespread. The transfer of information from neurons to glia occurs through the spillover helped by the existence of "tripartite synapses." Astrocytes can discriminate neuronal inputs of different origins and can integrate concomitant inputs (Volterra and Meldolesi 2005). Astrocytes integrate and process synaptic information elaborating a complex non-linear response to the incoming information from adjacent synapses.

In short, astrocytes integrate and process synaptic transmission and finally regulate synaptic transmission and plasticity, through the release of gliotransmitters (Lino et al. 2001). Astrocytes mainly signal through high-affinity slow-desensitizing receptors to modulate neurons and perform integration in spatiotemporal domains complementary to these neurons (Araque et al. 2014). According to Perea et al. (2008), astrocytes must, therefore, be considered an integral component of synaptic physiology.

2.4 Astrocytes and Neurovascular Regulation

The idea that astrocytes connect to blood vessels and neurons dates back to Camillo Golgi (1871) and beautifully illustrated by Ramón y Cajal (1895). However, the dynamic processes that complement these structural interactions, most notably the active dialogue between astrocytes and other elements of the central nervous system, have only begun to emerge recently. The functional networks of neuron, glia, and vascular cells have been termed the neurovascular unit. It is well known that neuronal activity leads to focal vasodilation, which is the basis of functional MRI (fMRI) studies (Klienfield et al. 1998; Raichle 1998). Tokano et al. (2006) are credited to be the first to demonstrate that astrocytic calcium elevation induces

vasodilation of the cortical perforating arterioles. Cajal (1895) hypothesized that constriction of astrocyte end-feet would trigger vasoconstriction and end-feet relaxation would induce vasodilation. About a century later, Paulson and Newman (1987) proposed astrocytic potassium "siphoning," i.e., influx of potassium ions into the astrocytes near active synapses and efflux of potassium from astrocyte to end-feet into the perivascular space and subsequently potassium-induced vasodilation as a mechanism of functional hyperemia.

Cellular imaging of neurons and astrocytes together with cerebral blood flow (CBF) recording in single vessels in vivo in living animals achieved only relatively recently using multiphoton microscopy of fluorescent-labeled blood vessels, and multicell bolus loading calcium indicators have helped in understanding the mechanism of functional hyperemia (Klienfield et al. 1998; Tokano et al. 2006). As a result of these studies, it has been possible to have a detailed dissection of different cellular components—blood vessels, astrocytes, pericytes, endothelium, and neurons—in vivo (Zonta et al. 2003; Haydon and Carmignoto 2006). This in turn is responsible for understanding brain energy metabolism.

2.5 Astrocytes and Brain Energy Metabolism

It is now well established that astrocytes play an active role in brain energy delivery, production, utilization, and storage (Allaman et al. 2011). While neurons consume nearly 20% of the oxygen and 25% of the glucose consumed by the human body, they generally lack mechanism for storing energy. There is close relationship between brain activity, glutamatergic neurotransmission, energy requirements, and glucose utilization (Belanger et al. 2011). As mentioned earlier, task-dependent increases in cerebral activity are accompanied by changes in local blood flow and glucose utilization. These processes are called "neurovascular" and "neurometabolic" coupling. Fox and Raichle (1986, 1988) in their PET studies established the mechanism underlying task-induced increase in glucose metabolism.

Astrocytes possess unique cytoarchitectural and phenotypic features that ideally positioned them to sense the surroundings and dynamically respond to changes in the microenvironment. They advance two different types of processes. On the one side, they constitute an essential component of the "tripartite synapse," and on the other, they are in contact with intraparenchymal microvasculature through their end-feet.

Astrocytes are thus tailored to ideally position themselves to sense neuronal activity at the synapses and respond to their appropriate metabolic supply via their end-feet (Belanger et al. 2011). Both astrocytes and neurons have a capacity to oxidize glucose and/or lactate. Enough evidence exists suggesting a role of astrocytes in coupling glutamatergic transmission and energy metabolism via a lactate shuttle (Suzuki et al. 2011). Astrocyte to neuron lactate transport is required for long-term memory formation. Astrocytes are also known to play an important role in the homeostasis of extracellular environment. They control the extracellular K+ concentration through the expression of specific channels. They play a critical

role in the clearance of glutamate from the synaptic cleft to terminate synaptic function (Araque and Navarrete 2010).

2.6 Astrocytes and Non-Neural Cells: Glia-Glia Interaction

Astrocytes also control non-neuronal brain cells. They attract cells to their territory through the release of chemokines. In this way, they coordinate the special positioning of microglia and synaptocytes during inflammation and oligodendroglia during development. They might drive reparative stem cells to lesion sites (Volterra and Meldolesi 2005). ATP releases cytokine leukemia-inhibiting factor (LIF) which promotes myelination activity of oligodendrocyte (Cohen and Fields 2008). Various factors released by astrocytes, e.g., PDGF, LIF, NT-3, NT-4, CNTF, and IGF-1, promote the differentiation, proliferation, and survival of oligodendrocyte precursor cells. These also help myelin formation and remyelination following injury (Gard et al. 1995).

2.7 Astrocytes and Neuroinflammation

Astrocytes are known to secrete membrane-associated molecules including cytokines, growth factors, and neurotransmitters (gliotransmitters) in response to physiological and pathological stimuli. It is therefore not surprising that they may have a role in nervous system disorders. Not only are they activated by these disorders, but they contribute to it (Ridet et al. 1977; Tandon 2007). One of the important roles astrocytes play in this respect is through participating in neuroinflammation. It has been known for a long time that following injury, damage, degeneration, and loss of neural tissue, there is proliferation of glial elements, particularly astrocytes, to replace it. However, their role in inflammation has been brought to light only recently (Tandon 2016; Tewari and Seth 2016). Like microglia, neuronal insult/damage also activates astroglia which then secrete a variety of cytokines and chemokines which contribute to neuroinflammation. The relative roles of microglia and astrocytes vary in different conditions. They promote angiogenesis, interaction with other extracellular molecules to regulate vascularization, and clearance of dying cells leading to a scar formation limiting the damaged area. On the other hand, scar arrests the growth of axons in the vicinity of the reactive astrocytes, thus stalling the regenerative process after injury. There is, thus, an active debate on the relative beneficial and detrimental aspect of astrocytes during neuroinflammation.

2.8 Astrocytes and Other Neuropathological Conditions

It is now known that astrocytes play an important role in the clearance of glutamate. The glutamate-induced neurotoxicity is blamed for the pathogenesis of a variety of

neurological disorders, e.g., epilepsy, trauma, stroke, and even neurodegenerative disorders. Astrocytes failing to clear excessive glutamate are responsible for their involvement in these pathologies. Tewari and Seth (2016) in their Table 3.2 have provided a summary of "astrocyte disorders" which include Alzheimer's, Huntington's, and Parkinson's diseases and also epilepsy, autism, multiple sclerosis, and others. It provides information on the nature of astrocyte response in each of these disorders and a list of important recent references. Chapters 8, 9, 10, 11 and 12 are also dealing with the role of astrocytes in brain disorders.

In their extensive review, Verkhratsky and Nedergard (2018) have provided information on some little-known functions of astroglia. These include relation to chemoreception of oxygen, CO_2, and pH and regulation of respiration and circadian rhythm. Their role in higher cognitive function has been postulated.

In summary, astrocytes are highly heterogeneous in form and function. They are intimately integrated into the neural network and control CNS homeostasis at all levels of organization from molecular to the whole organ. Astrocytes play an important role in brain development, neuronal differentiation and guidance, and neurite outgrowth. Recent studies have shown that astrocytes not only regulate neurogenesis but are also the neuronal progenitors. They play an active role in the brain energy production, delivery, utilization, and storage. Astrocytes are chemosensing elements of the brain contributing to systemic homeostasis of ions, metabolites, and energy. Till recently considered non-excitable, they are now known to be excitable primarily regulated by Ca2+ levels in the cytoplasm. They are endowed with a large number of ion channels, neurotransmitters and neuromodulator receptors, and SLC transporters. Astrocytes have been found to secrete a host of molecules (gliotransmitters) which control astrocyte to neuron communication and also synaptic transmission. It is now universally acknowledged that astrocytes modulate both the intrinsic neuronal excitability and the strength of synaptic transmission. The neurovascular unit constituted by the astrocytes provided for focal vasodilation in response to neuronal activity, i.e., the functional hyporemia and neuroinflammation. Disturbances in these diverse physiological functions of astrocytes are therefore closely related to diverse neuropathological disorders and neurodegeneration.

3 Biology of Glia: Part II—Microglia

3.1 History

First recognized by Franz Nissl (1860–1918) during his studies on the histopathology of dementia as the rod cells in 1880, it was Pio del Río Hortega (1882–1945) who along with his student Collado in 1918 using his silver staining technique illustrated the detailed morphology of microglia. According to one of Hortega's biographers, "The microglia danced to him and revealed their graceful limbs ------ under the microscope he found a world of beauty which pleased his artistic soul and satisfied his inquisitive mind." He described microglia as a unique cell type differing

in morphology from other glia and neurons (Del Río-Hortega 1919; Sierra et al. 2016).

Ramón y Cajal, who provided a detailed description of astrocytes in the late 1890s and early 1900s, found evidence for some of other cells (other than neurons and astrocytes) which did not stain as well by his famous gold chloride sublimate method and called them the "third element." Río Hortega, who considered Cajal as his mentor and master, modified the staining techniques such as the ammoniacal silver carbonate and demonstrated that the "third element" of Cajal consisted of two distinct cell types, i.e., microglia (mesoglia) and oligodendrocyte (which he first called interfascicular glia). According to Sierra et al. (2016), "Surprisingly, however, the field of microglia did not advance for decades and some neuropathologists even denied their existence for most part of the twentieth century. Only in late 1960s Georg Kreutzberg started to study these cell types again. This marked a rebirth of microglia research"

3.2 Morphology

Microglia constitute approximately 15–20% of the total glial population (Carson et al. 2006). They are not ectodermal in origin and were believed to be of mesenchymal lineage (Barron 1995). It is remarkable that Hortega in one of his papers on "Putative Origin of Microglia" in 1919 provided a detailed reasoning "to believe that microglia histogenetically differ from ordinary astrocytes and that their nature is mesodermal."

Morphologically resting and activated microglia manifest different characteristics. The former are characteristically elongated cell bodies with spine-like processes that often branch perpendicularly. Nimmerjahn et al. (2005) have demonstrated that even in the resting state, while the soma and main branches remained stable for hours, their processes were remarkably motile, undergoing cycles of formation of extension and withdrawal on time scale of minutes. In contrast, in the activated state, the cell body increases in size; there is a thickening of proximal processes. These processes were observed to directly contact astrocytes, neuronal cell bodies and blood vessels, decrease in ramification of distal branches suggesting that in healthy brain microglia serve some house-keeping function (Fetler and Amigorena 2005).

The morphological and structural evolution of microglia in pathological conditions creates a variety of cell types. When they migrate to the site of injury to perform their phagocytic function, they undergo a hypertrophic transformation and acquire multiple shapes and elongated forms becoming rod cells: when they engulf damaged elements, they become granuloadipose bodies (Del Río-Hortega 1919). This activation is believed to be preceded by molecular events like changes in their expression of cell adhesion molecules, cytoskeleton reorganization, and antigen presentation (Patro and Patro 2004).

Microglia play an important role during the development of the CNS (see below). During this period, they manifest different morphologies, i.e., the ameboid form

which originates from the yolk sac. They acquire a round or irregular shape (Pont-Lezica et al. 2011). This is in contrast to the ramified "or" resting surveillant microglia of adult CNS and the "reactive" or "activated" microglia.

3.3 Distribution

All brain regions contain microglia in varying numbers, but they are more abundant in the gray matter. Microglia interact more or less closely with neurons, both protoplasmic and fibrous astrocytes, and the blood vessels (Graber et al. 2016). Recently, a special type of microglia with close association to neurons has been described by Baalman et al. (2015).

3.4 Functions

Microglia are the immunocompetent resident macrophages of the CNS, responsible for innate immunity. They kill invading microorganisms in the brain, remove debris, and facilitate tissue repair after injury. During development of the brain, they play a major role in the developmental pruning of unnecessary synapses (Rakic and Zecevic 2000). In the adult brain, the "ramified" microglia are really not resting but continuously survey the healthy brain for any damage as shown by in vivo time-lapse video microscopy by Nayak et al. (2014). They serve an immune-surveillance function and can sense subtle changes in the microenvironment through a variety of surface receptors (Barron 1995; Nimmerjahn et al. 2005). Such microglia release various neurotrophic growth factors to promote the neuronal survival and also enhance neurogenesis. During an injury or degeneration to the brain, the microglia get activated and release neuroinflammatory molecules, growth factors, matrix proteins, chemokines, prostaglandins, and reactive free radicals (Patro et al. 2016; Streit 2005; McGeer and McGeer 2001).

Microglia in Immune Regulation

As mentioned earlier, microglia when activated provide innate immunity to the CNS. Such activated microglia release a number of immunocompetent molecules and chemokines like macrophage inflammatory protein 1α (M1P1α), monocyte chemoattractant protein 1 (MCP1), IL, 1 L/β, IL3, IL6, IL10, IL12, IL15, IL18, and tumor necrosis factor-α. These molecules not only control inflammation but also regulate immune response of the brain. At the same time, activated microglia also promote neuroprotection by releasing anti-inflammatory and growth factors like NGF, BDNF, and NT-3. The relative role of microglia in neuronal damage and protection depends upon a variety of factors like the nature of damage and pathology, the duration of insult (acute or chronic), and the age of the patient (Suzuki et al. 2004; Colton 2009).

Role of Microglia in Neuroinflammation and Diverse Neuropathologies

Microglia, the resident macrophages of the nervous system, play a dominant role in the pathophysiology of neuroinflammation, a common feature of brain pathologies, from the moment of an insult or injury, damage, or destruction to the neural tissue. Microglia invade the affected region and get activated. Even in their resting stage, they serve an immune-surveillance function. They can sense subtle changes in the microenvironment through a variety of highly conserved pattern recognition surface receptors (Barron 1995; Nimmerjahn-et al. 2005). Like all toll-like receptors (TLRs) to recognize both pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs). On the other hand, activated microglia are capable of releasing a variety of pro-inflammatory factors like NO, H_2O_2, OH, NOO, TGFX3, and PGE2 and a variety of interleukins (Streit et al. 1999; Block and Hong 2005; Tandon 2007). While the role of microglia in acute insult to the brain was well known, it is only recently that their role in neuroinflammation associated with neurodegeneration has been brought to light (Block and Hong 2005; Brown and Neher 2010). Patro et al. (2016) in their paper (Table 2.1) have summarized the neurodegenerative and neuroprotective role of microglia in various pathological conditions which include AD, PD, HD, ALS, MS, cerebral ischemia, prion disease, HIV, AIDS, brain tumor, and chronic pain.

While the neurotoxic or destructive role of microglia is well known, activated microglia also act as a defense mechanism for various insults to the brain. They kill invading microorganisms, remove debris, and facilitate tissue repair after injury. Being the immune cell of the CNS, they protect and repair the damage and also facilitate the healing process (Alosi 2001; Ekdahl et al. 2009). Apart from their conventional neuromodulatory function, they contribute to neuroendocrine regulation as well as neurogenesis (Chan et al. 2007; Ghosh and Ghosh 2016).

4 Biology of Glia: Part III—Oligodendrocytes

4.1 History

Oligodendrocytes, now well recognized as the myelinating cells of the central nervous system, were first defined by Río Hortega as a distinct entity—a constituent of the "third element" of his mentor Ramón y Cajal whose staining technique had failed to reveal them. Working in Hortega's laboratory, Penfield succeeded in developing an exquisite picture of these cells. As mentioned in his autobiography, "No Man Alone," "One morning, I was thrilled to see that 'Oligo cells' in one of my sections were especially clear, complicated and beautiful. The cells were not 'few-branching' but many branching '---------'. Then I stood up and handed a section to don Pio. Finally, he turned and said quietly-----. 'Casi mejor quo Yo' (Almost better than I could do)." On the advice of Hortega, this was published in *Brain* by Penfield (1924).

4.2 Origin and Development of Oligodendrocytes

Studies of the human fetal forebrain suggest that human oligodendrocytes have multiple origins. Simultaneous presence of three different populations which give rise to oligos has been reported (Jakovcevski and Zecevic 2005). However, it is not known whether oligodendrocytes from these different sources have different roles, myelinate different axonal pathways, or affect the outcome of CNS pathologies (Bradl and Lassmann 2010). Most of the earlier studies on oligodendrocyte development and myelin formation were on rodents. Even though there are some differences between the rodent and human oligodendrocytes, there is a great deal of similarity. They are all derived from oligodendrocyte progenitor cells (OPCs) which arise from the medial ganglionic eminence and anterior endopeduncular area of the ventral forebrain. These OPCs populate the entire embryonic telencephalon including the cerebral cortex (Bradl and Lassmann 2010). There are several waves of these cells, and they have to travel long distances in order to end up in their final place of destination. The migration is guided by regulation signals like PDGF, FGF, netrins, semaphorins, and chemokines CXL. Once located at their final destination, some OPCs persist into adulthood, while the vast majority differentiate to myelin-producing oligodendrocytes (Kuhn et al. 2019).

Rogister et al. (1999) provided a detailed account of the origin of OPCs from neural stem cells through the developing CNS. Jakovcevski and his colleagues have elaborated the sequence of oligodendrocyte development in human fetal telencephalon (Jakovcevski and Zecevic 2005; Jakovcevski et al. 2009). There are some differences between the OPCs distributed in white and gray matter, being evenly distributed in the former and being less abundant in the latter.

Recent molecular biological investigations have revealed distinct markers for various stages of development of OPCs and embryonic and adult oligodendrocytes (Kuhn et al. 2019).

4.3 Oligodendrocytes and Myelination

The key function of oligodendrocytes is myelination of the axons in the CNS. The myelin sheath is an extension of oligodendrocyte (and Schwann cell) plasma membrane that wraps around axons in concentric fashion. In the CNS, oligodendrocytes myelinate large diameter axons and provide trophic support for the underlying axons (Kuhn et al. 2019; Simons and Trajkovic 2006). Myelination is a complex and highly regulated process. Myelination occurs as a result of the upregulation of myelin protein expression which leads the number of wraps around the axon. With time, the number of wraps increases, thereby forming compact myelin internodes. Throughout the process, more oligodendrocytes are produced than necessary. The extras undergo apoptosis. The final number of oligos that survive matches the number and length of axons that need to be myelinated (Barres and Raff 1999; McTigue and Tripathi 2008). According to Barres and Raff (1993), although oligos themselves do not divide, the proliferation of oligodendrocyte precursor cells

(OPCs) that give rise to them does depending upon the electrical activation of the neighboring axons. They observed that axonal electrical activity normally controls factors that are responsible for the proliferation of OPCs and thereby helps to control the numbers of oligos that develop in the region (for details, see Gibson et al. 2014). It has been shown that axons do so through regulating astrocytes, but not OPC proliferation. Furthermore, oligos survival depends on the release of PDGF, IGF-1, CNTF, or NT-3 by astrocytes (Barre and Raff 1993, 1999; Barre et al. 1993). In addition, axonally derived neuregulin (NRG) is a likely candidate signal that mediates axonally promoted survival of mature myelinating oligodendrocytes. For all practical purposes, Schwann cells in the peripheral nervous system behave in the same manner (but this is not being discussed in any details here).

Oligodendrocytes not only ensheath axons to electrically insulate these structures but also induce a clustering of sodium channels along the axon, at the node of Ranvier, which is one important prerequisite for saltatory nerve conduction (Bradl and Lassmann 2010). Molecular signals that initiate CNS myelination are still ill understood. However, they provided evidence that the onset of CNS myelination in normal development might be determined by the degree of neuronal differentiation and not by the timing of an intrinsic oligodendrocyte differentiation program. It is now well established that there is a cross-talk between the neurons and oligodendrocytes for the initiation of myelination. Electrical activity is believed to control the release of PDGF (platelet-derived growth factor) by the neurons which enhances mitosis of OPCs. On the other hand, myelin- forming cells also send essential signal to axons. Expression of myelin genes such as myelin-associated glycoprotein (MAG) and PLP appears necessary for axonal function and survival throughout life (Rogister et al. 1999; Yin et al. 1988). Although the electrical activity of neurons in the CNS is an essential promyelinating factor, additional changes on neurons seem to be needed to drive efficient myelin formation (Barres 2008; Bradl and Lassmann 2010).

There is increasing evidence that cells of the oligodendrocyte linage are capable of responding to a variety of neurotransmitters. Glutamate released by axons of glutamatergic neurons could be a regulator of OPC numbers (Yuan et al. 1998). Similarly, expression of non-NMDA Kainate, AMPA-preferring glutamate receptors, adrenergic receptors and several others by OPC possibly regulate their proliferation and differentiation. This has been extensively reviewed by Araque et al. (1999), Rogister et al. (1999), Mc Tigue and Tripathi (2008) in their Table 1 provide a long list of positive and negative effect of different molecules on OPC proliferation and differentiation.

4.4 Non-Myelinating Functions of Oligodendrocytes and OPCs

In addition, another player in the early axon/oligodendrocyte cross-talk is Jagged, a ligand that signals notch 1 receptor on OPC and inhibits oligodendrocyte differentiation (Wang et al. 1998). Besides, the primary function of myelination oligodendrocytes and OPCs has recently been shown to have immunomodulatory

capacity. OPCs express cytokine receptors and assess their microenvironment through filopodia extension (Kuhn et al. 2019). According to Kirby et al. (2019), OPCs present antigen and are cytotoxic targets in inflammatory demyelination. In response to inflammatory cues, OPCs, like microglia, have been shown to migrate to the site of injury. Their precise role in this regard needs to be explored further (Kirby et al. 2019). In short, oligodendrocytes are now recognized as critical regulators of neuronal function in CNS development, homeostasis, and regeneration (Kuhn et al. 2019).

Oligodendrocytes and the myelin sheath metabolically support axons. According to Lee et al. (2012), there is enough evidence to suggest that oligodendroglia support axon survival through a myelin-independent mechanism. Oligodendrocytes can provide trophic support for neurons by the production of neurotrophic factors like GDNF, BDNF, and insulin-like growth factor 1 (IGF1) (Du and Dreyfus 2002). Oligos can generate lactate, which can then be transferred to axons to generate metabolic energy in the form of ATP (Bercury and Macklin 2015). This is done with the help of (monocarboxylate transporter 1) MCT 1 lactate transporter which is localized to oligodendrocytes in vivo. In addition, a number of glycolytic and Krebs cycle enzymes contribute to glucose metabolism and ATP production (Kuhn et al. 2019; Lee et al. 2012; Pierre and Pellerin 2005).

4.5 Oligodendrocytes and CNS Pathology

A combination of high metabolic rate with its toxic by-products, high intracellular iron and low concentration of the antioxidative glutathione, oligodendrocytes are particularly vulnerable to oxidative damage and mitochondrial injury. Hence, the oxidative damage is a common contribution to the oligodendrocyte loss under many pathological conditions like MS, ischemia and injury, and inflammation and infection. Oligodendrocytes also express a variety of molecules which make it susceptible to excitotoxic cell death, glutamate toxicity, and damaging effect of extracellular ATP. Oligodendrocyte loss can also occur as a result of exposure to inflammatory chemokines like tumor necrosis factor-α (TNFα) (Bradl and Lassmann 2010; Barres 2008; Jana and Pahan 2006; Jurewicz et al. 2005).

Oligodendrocytes are easily destroyed by specific autoantibodies as seen in patients with MS-like inflammatory demyelination diseases. As a matter of fact, demyelination and oligodendrocyte death are common features of inflammatory white matter lesions as Devic's disease (neuromyelitis optica) and autoimmune encephalomyelitis.

The above pathogenetic mechanisms may damage oligodendrocytes alone or in association with damage to the myelin also. It has been observed that different pathological patterns of white matter injury reflect different mechanisms of myelin and oligodendrocyte damage. Primary oligodendrocyte injury is seen in conditions of infections such as progressive multifocal encephalopathy. In contrast, combined demyelination and oligodendrocyte damage is observed in ischemic lesions of the white matter and stroke and also in severe inflammatory brain lesions as seen in

acute multiple sclerosis, virus encephalitis like herpes simplex encephalitis, and progressive multifocal encephalomyelitis (Aboul-Enein et al. 2003; Bradl and Lassmann 2010). Increased number of oligodendrocytes, unassociated with demyelination, has been reported a couple of days after spinal contusion. This remarkable amount of oligogenesis occurs in a gliogenic zone along the borders of spinal contusion lesion (Tripathi and Mc Tigue 2007; Mc Tigue and Tripathi 2008). Oligodendrocyte genesis has also been observed along the borders of ischemic lesion in the brain where the new cells are co-localized with astrocytes, microglia, and macrophages (Mabuchi et al. 2000).

In addition to the acute lesions of the CNS mentioned above, oligodendroglial pathology is also reported in several neurodegenerative disorders. Rarely genetic defects that lead to oligodendrocyte damage are reported in some leukodystrophies; this is due to the accumulation of mutated PLP1 (Torii et al. 2014). White matter pathology is a characteristic of Alzheimer's disease (AD) (Fischer et al. 2015; Desai et al. 2010). However, oligodendrocytes and demyelination are believed to occur secondary to degeneration (Xu et al. 2001). Oligodendrocyte pathology can be evident even before any neurodegenerative event materializes (Fischer et al. 2015).

A reduction of perineuronal oligodendrocytes in gray matter of prefrontal cortex has been reported in schizophrenia (SZ). This is specially so in the left CA4 region of the anterior and posterior hippocampus. This decreased number of oligodendrocytes was found to be associated with cognitive deficit. In addition, Raabe et al. (2019) have demonstrated that expression of myelin and oligodendrocyte-related genes was profoundly affected in the prefrontal, temporal, and occipital cortex, hippocampus, and basal ganglia (for further details, also see Cassoli et al. 2015; Uranova et al. 2007; Vikhreva et al. 2016; Schmitt et al. 2015). Lee et al. (2012) demonstrated that oligodendrocyte-specific MCT 1 (monocarboxylate transporter 1) loss causes axonopathy. Similarly, the reduced ability of gray and white matter oligodendroglia to support motor neurons caused by altered MCT1 expression may contribute to amyotrophic lateral sclerosis (ALS) pathogenesis.

Oligodendrocytes are the cells of origin of both oligodendrogliomas and oligoastrocytomas. On the basis of an immunohistological and electron microscopic study of 55 such tumors, Sarkar et al. (1988) observed that both these tumors arise from a common progenitor cell capable of differentiation into both oligodendrocyte and astrocyte. The nature and degree of differentiation depend probably on gene expression and/or some microenvironmental factors. Oligodendrogliomas express S100, MAP 2, and other markers; IDH1 (R132H) is uniformly positive in the majority of oligodendrogliomas. But there is no specific immunohistochemical marker for the diagnosis.

More than 90% of oligodendrogliomas harbor IDH1 mutation. Concurrent deletion of chromosomal arms 1P and 19q is the diagnostic alteration. Most common mutation in IP/19q co-deleted oligodendrogliomas is present in CIC gene on 19q.13.2 followed by FUBP1 mutation on 1p 31.1. Prognostically favorable role of IDH1 mutation and 1P/19Q co-deletion is noted in those who are treated with adjuvant radiotherapy and/or chemotherapy in contrast to those treated with surgery alone (Rao and Santosh 2018).

Advances in knowledge about the structure and function of glial cells have a major impact on our understanding of normal neural function and pathogenesis of a variety of brain diseases, specially neuroinflammation and neurodegeneration.

References

Aboul- Enein F, Rauschka H, Kornek B, Stadelmann C, Stefferl A, Brück W et al (2003) Preferential loss of myelin-associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain disease. J Neuropathol Exp Neurol 62(1):25–33

Allaman I, Belanger M, Magistretti PJ (2011) Astrocyte-neuron metabolic relationships for better and for worse. Trends Neurosci 34(2):76–87

Aloisi E (2001) Immune function of microglia. Glia 36(2):165–179

Andriezen I (1893) The neuroglial elements in human brain. Brit Med J 2:227–230

Araque A, Carmingnoto G, Haydon PG (2001) Dynamic signaling between neurons and glia. Ann Res Physiol 63(1):795–813

Araque A, Carmingnoto G, Haydon PG (2014) Gliotransmitters travel in time and space. Neuron 81(4):728–739

Araque A, Navarrete M (2010) Glial cell in neuronal network function. Philos Trans R Soc Lond B Biol Sci 365(1551):2375–2381

Araque A, Parpura V, Sanzgiri RP, Haydon PG (1999) Tripartite synapses: glia: the unacknowledged partner. Trends Neurosci 22(5):208–215

Baalman K, Marin MA, Ho TS, Godoy M, Cherian L, Robertson C et al (2015) Axon initial segment associated microglia. J Neurosci 35(5):2283–2292

Barre BA, Jacobson R, Schmidt M, Sendtner M, Raff MC (1993) Does oligodendrocyte survival depend on axons? Curr Biol 3(8):489–497

Barres BA (2008) The mystery and magic of glia: a perspective on their role in health and diseases. Neuron 60(3):430–440

Barres BA, Raff MC (1993) Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. Nature 361(6490):258–260

Barres BA, Raff MC (1999) Axonal control of oligodendrocyte development. J Cell Biol 147(6): 1123–1128

Barron KD (1995) The microglia cell: a historical review. J Neurol Sci 34:57–68

Bellanger M, Allaman L, Magistretii PJ (2011) Brain energy metabolism: focus on astrocyte neuron metabolic cooperation. Cell Metab 14(6):724–738

Bercury KK, Macklin WB (2015) Dynamics and mechanism of CNS myelination: dev. Cell 32(4): 447–458

Bezzi P, Volterra A (2001) A neuron –glia signaling network in the active brain. Curr Opin Neurobiol 11(3):387–394

Block ML, Hong JS (2005) Microglia and inflammation mediated neurodegeneration multiple triggers with a common mechanism. Prog Neurobiol 76(2):77–98

Bolton MM, Eroglu C (2009) Look who is weaving the neural web: glial control of synapse formation. Curr Opin Neurobiol 19(5):491–497

Bradl M, Lassmann H (2010) Oligodendrocytes: biology and pathology. Acta Neuropathol 119(1): 37–53

Brown GC, Neher JJ (2010) Inflammatory neurodegeneration and mechanism of microglial killing of neurons. Mol Neurobiol 41(2):242–247

Bushong EA, Martone ME, Jones YZ, Ellisman MH (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J Neurosci 22(1):183–192

Cajal, Ramón Y (1897) Algo Sobre La Signification de la neuroglia (Something about the physiological significance of neuroglia)

Cajal Ramón Y (1913) Contribution al conocimante de la neuroglia del cerebro humano. A contribution to the understanding of neuroglia in human brain (quoted by Navarrete and Araque 2014)
Cajal Ramón YS (1895) Algunas conjeturas sobre el mecanismo anatómico de la ideación, asociación y atención. Rev Med Cirug Pr c 36:3–14
Carmingnoto G (2000) Reciprocal communication systems between astrocytes and neurons. Prog Neurobiol 62(6):561–581
Carson MJ, Thrash CJ, Walter B (2006) The cellular response in neuroinflammation: the role of leukocytes, microglia and astrocytes in neuronal death and survival. Clin Neurosci Res 6(5): 237–245
Cassoli JS, Guest PC, Malchow B, Schmitt A, Falkai P, Martins-de-Souza D et al (2015) Distributed macro-connectivity in schizophrenia linked to oligodendrocyte. NPJ Schizophr 1: 15034
Chan WY, Kohasaka C, Rezaic P (2007) The origin and cell lineage of microglia: new concepts. Brain Res Rev 53(2):344–354
Cohen JE, Fields RD (2008) Activity dependent neuroglial signalling by ATP and leukaemia inhibitory factor promotes hippocampal glial cell development. Neuron Glia Biol 4(1):43–55
Colton CA (2009) Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol 4(4):399–418
Del Río-Hortega P (1919) El "tercer elemento" de los centrosnerviosus. Bol Soc Esp Biol 9:69–120
Del Río-Hortega P (1920) La microglia su transformacionen celulas en baston cito Y cerpos granulo adiposos. Trab Lab Invest Biol Madrid 18:37–82. Quoted by Kuhn S, Gritti L, Crooks D, Dombrowski Y (2019) Oligodendrocytes in development, myelin generation and beyond. Cell 8(11):1424
Del Río-Hortega P (1932) Microglia. In: Penfield W (ed) Cytology and cellular pathology of the nervous system. Hoeber, New York, pp 481–584
Desai MK, Mastrangelo MA, Ryan DA, Sudol KL, Narrow WC, Bowers WJ (2010) Early oligodendrocytes/myelin pathology in Alzheimer's disease mice constitutes a novel therapeutic target. Amer J Pathol 177(3):1422–1436
Du Y, Dreyfus CF (2002) Oligodendrocytes as providers of growth factors. J Neurosci Res 68(6): 647–654
Ekdahl CT, Koike MA, Lindvall O (2009) Brain inflammation and adult neurogenesis the dual role of microglia. Neuroscience 158(3):1021–1029
Emsley JG, Macklis JD (2006) Astroglial heterogeneity closely reflects the neuronal-defined anatomy of the adult murine CNS. Neuron Glia Biol 2(3):175–186
Farmer WT, Abrahamson T, Sabrina C (2016) Neurons diversify astrocytes in the adult brain through sonic hedgehog signaling. Science 351(6275):849–854
Fetler L, Amigorena S (2005) Brain under surveillance: the microglia patrol. Science 309(5733): 392–393
Fischer FU, Wolf D, Scheurich A, Fellgiebel A (2015) Altered whole-brain white matter networks in preclinical Alzheimer's disease. Neuro Image Clin 8:660–666
Fox PT, Raichle ME (1986) Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. PNAS 83(4):1140–1144
Fox PT, Raichle ME (1988) Nonoxidative glucose consumption during focal physiologic neural activity. Science 241(4864):462–464
Gallo V, Chittajallu R (2001) Unwrapping glial cells from the synapse: what lies inside? Science 292(5518):872–873
Gard AL, Burrell MR, Rudge JS, Williams WC (1995) Astroglial control of oligodendrocyte survival mediated by PDGF and leukemia inhibitory factor like protein. Development 121(7): 2187–2197
Ghosh P, Ghosh A (2016) Role of microglia in adult neurogenesis. In: Jana N, Basu A, Tandon PN (eds) Inflammation: the common link in brain pathologies. Springer, Singapore, pp 325–345

Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS et al (2014) Neuronal activity promotes oligodendrogenesis and adaptive myelination in mammalian brain. Science 344(6183):1252304

Golgi C (1885) Sulla fina anatomia degli organi centrali del sistema nervoso. Vlll. Tessuto interstiziale degli organi nervosi centrali (Neuroglia). Riv Sper Freniat 11:72–123

Grabert K, Michoel T, Karavolos MH, Clohisey S, Baillie JK, Stevens MP et al (2016) Microglial brain region− dependent diversity and selective regional sensitivities to aging. Nat Neurosci 19(3):504–516

Haydon PG (2001) Glia listening and talking to the synapse. Nat Rev Neurosci 2(3):185–193

Haydon PG, Carmingnoto G (2006) Astrocyte control of synaptic transmission and neurovascular coupling. Physiol Rev 86(3):1009–1031

Jakovcevski I, Fillipovic R, Mo Z, Rakic S, Zecevic N (2009) Oligodendrocyte development and the onset of myelination in human fetal brain. Front Neuroanat 3:1–15

Jakovcevski I, Zecevic N (2005) Sequence of oligodendrocyte development in the human fetal telencephalon. Glia 49(4):480–491

Jana A, Pahan K (2006) Oxidative stress kills human primary oligodendrocyte via neural sphingomyelinase: implications for multiple sclerosis. J Neuroimmune Pharmacol 2(2): 184–193

Jurewicz A, Maiysiak M, Tybor K, Kilianek L, Raine CS, Selmaj K (2005) Tumour necrosis factor-induced death of adult human oligodendrocyte is maintained by apoptosis inducing factor. Brain 128(11):2675–2688

Kettenmann H, Verkhratshy A (2008) Neuroglia: 150 years after. Trends Neurosci 31:653–659

Khakh BS, Deneen B (2019) The emerging nature of astrocyte diversity. Annu Rev Neurosci 42: 187–207

Khakh BS, Sofroniew MV (2015) Diversity of astrocytes functions and phenotypes in neuronal circuits. Nat Neurosci 18(7):942–952

Kirby I, Jin J, Cardona JG, Smith MD, Martin KA, Wang J et al (2019) Oligodendrocyte precursor cells present antigen and are cytotoxic targets in inflammatory demyelination. Nat Commun 10(1):1–20

Klienfeld D, Mitra PP, Helmchen F, Denk W (1998) Fluctuations and stimulus-induced changes in blood flow observed in individual capillaries in layer 2 through 4 of rat neocortex. PNAS 95(26):15741–15746

Kuffler SW (1967) Neuroglia cells: physiological properties and a potassium mediated effect of neuronal activity on the glial membrane potential. Proc R Soc B 168(1010):1–21

Kuhn S, Gritti L, Crooks D, Dombrowski Y (2019) Oligodendrocyte in development, myelin generation and beyond. Cell 8(11):1424

Lee Y, Morrison BM, Li Y et al (2012) Oligodendroglia metabolically supports axons and contribute to neurodegeneration. Nature 487(7408):443–448

Lenhossek MV (1895) Der feiner Bau des Nervensystems im Lichte neuester Forschungen, 2nd Ed., Fischer's Medicinische Buchhandlung H, Kornfeld (Quoted by Matyash and Kettenmann 2010)

Lino M, Goto K et al (2001) Glia synapse interaction through $Ca2^{+}$ permeable AMPA receptors in Bergmann glia. Science 292(5518):926–929

Mabuchi T, Kitagawa K, Ohtsuki T, Kuwabara K, Yagita Y, Yanagihara T et al (2000) Contribution of microglia macrophages in expansion of infarction and response of oligodendrocytes after focal cerebral ischemia in rats. Stroke 31(7):1735–1743

Matyash V, Kettenmann H (2010) Heterogeneity in astrocyte morphology and physiology. Brain Res Rev 1(63):2–10

Mc Tigue DM, Tirpathi RB (2008) The life death and replacement of oligodendrocytes in the adult CNS. J Neurochem 107(1):1–9

McGeer PL, McGeer EG (2001) Inflammation, autotoxicity and Alzheimer's disease. Aging 22(6): 799–809

Navarrete M, Araque A (2014) The Cajal School and the physiological role of astrocytes: a way of thinking. Front Neuroanat 8:1–5
Nayak D, Roth TL, Mc Gaven DB (2014) Microglia development and function. Annu Rev Immunol 32:367–402
Newman EA (2003) New roles for astrocytes: regulation of synaptic transmission. Trends Neurosci 26(10):536–542
Newman EA, Araque A, Dubinsky JM (2017) The beautiful brain. The Drawing of Santiago Ramón Y Cajal Abrams
Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318
Oberheim NA, Tokano T, Han X, He W, Lin JH, Wang F et al (2009) Uniquely hominid features of adult human astrocytes. J Neurosci 29(10):3276–3287
Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29(10):547–553
Oliet SHR, Piet R, Poulain DA (2001) Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. Science 292(5518):923–925
Patro I, Nagayach A, Sinha S, Patro N (2016) General physiology and pathophysiology of microglia during neuroinflammation. In: Jana N, Basu A, Tandon PN (eds) Inflammation: the common link in the brain pathologies. Springer, Singapore, pp 43–70
Patro IK, Patro N (2004) Effect of immuneactivator (poly I:C) on the rat cerebral cortex. J Tissue Res 3(2):71–75
Paulson OB, Newman EA (1987) Does the release of potassium from astrocyte end feet regulate cerebral blood flow? Science 237(4817):896–898
Penfield W (1924) Oligodendroglia and its relation to classical neuroglia. Brian 47:430–452
Penfield WP (1928) Neuroglia and microglia. In: Cowdry EV (ed) Special cytology. Hoeber, New York
Perea G, Araque A (2006) Synaptic transmission processing by astrocytes. J Physiol Paris 99:92–97
Perea G, Araque A (2010) Glia modulates synaptic transmission. Brain Res Rev 1(63):93–102
Perea G, Navarrete M, Araque A (2008) Tripartite synapse: astrocytes process and control synaptic information. Trends Neurosci 32(8):421–431
Pierre K, Pellerin L (2005) Monocarboxylate transporters in the central neurons system: distribution, regulation and function. J Neurochem 94(1):1–14
Pont-Lezica L, Bechade C, Cantaut-Belarif Y, Pascual O (2011) Physiological roles of microglia during development. J Neurochem 119(5):901–908
Raabe FJ, Slapakova L, Rossner MJ, Cantuti-Castelvetri L, Simons M, Falkai PG et al (2019) Oligodendrocytes as a new therapeutic target in schizophrenia from histopathological findings to neuron- oligodendrocytes interaction. Cell 8(12):1496
Raichle ME (1998) Behind the scenes of functional brain imaging: a historical perspective. PNAS 95(3):765–772
Rakic S, Zecevic N (2000) Programmed cell death in developing telencephalon. Eur J Neurosci 12(8):2721–2734
Rao S, Santosh V (2018) Diffuse astrocytic and oligodendroglial tumors. In: Sarkar C, Santosh V, Chacko G (eds) Essentials of diagnostic surgical neuropathology. Medical and Scientific Publishers, Noida, pp 25–30
Retzius G (1894) Die neuroglia des Gehirnbeim Menschen and bec saeugethieren. Biol Untersuchungen 6(1):28
Ridet J, Privat A, Malhotra SK, Gage FH (1977) Reactive astrocytes: cellular and molecular cues to biological function. Trends Neurosci 20(12):570–577
Robertson M (2014) Astrocytes and the evolution of the human brain. Med Hypothesis 82(2): 236–239
Rogister B, Ben- Hur T, Dubois-Dalcq M (1999) From neural stem cells to myelinating oligodendrocytes. Mol Cell Neurosci 14(4–5):287–300

Santello M, Toni N, Volterra A (2019) Astrocyte function from information processing to cognitive impairments. Nat Neurosci 22(2):154–166
Sarkar C, Roy S, Tandon PN (1988) Oligodendroglial tumors: an immunohistochemical and electron-microscopic study. Cancer 61(9):1862–1866
Schmitt A, Malchow B, Keeser D, Falkai P, Hasan A (2015) Neurobiology of schizophrenia: new findings from the structure to the molecules. Nervenarzt 86(3):328–331
Sierra M, De Castro F, Río-Hortega, Del J, Garrosa M (2016) The "Big-Bang" for modern glial biology: Translation and comment on Pio del Río Hortega 1919 series of papers on microglia. Glia 64(11):1801–1840. Contains English translation of four papers on microglia by Río Hortega published in 1919 in a Spanish Journal
Simons M, Trojkovic K (2006) Neuron-glia communication in control of oligodendrocyte function and myelin biogenesis. J Cell Biochem 119(21):4381–4389
Somgen GG (1988) Nervenkitt: notes on the history of concept of neuroglia. Glia 1(1):2–9
Streit WJ (2005) Microglia and neuroprotection: implications for Alzheimer's disease. Brain Res Rev 48(2):234–239
Streit WJ, Walter SA, Pennell NA (1999) Reactive microgliosis. Prog Neurobiol 57(6):63–81
Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. Cell 144(5):810–823
Suzuki T, Hide I, Ido K, Kohsaka S, Inoue K, Nakata Y (2004) Production and release of neuroprotective tumor necrosis factor by P2X7 receptor activated microglia. J Neurosci 24(1): 1–7
Tandon PN (2007) Brain cells- recently unveiled secrets: their clinical significance. Neurol India 55(4):322–327
Tandon PN (2016) Biology of neuroinflammation: a common denominator in brain pathology. In: Inflammation: the common link in brain pathologies (eds) N Jana, A Basu, Tandon PN. Springer, Singapore 1–16
Tewari M, Seth P (2016) Astrocytes in neuroinflammation and neuronal disorders: shifting the focus from neurons. In: Jana N, Basu A, Tandon PN (eds) Inflammation: the common link in the brain pathologies. Springer, Singapore, pp 43–70
Tokano T, Tian GF, Peng W, Lou N, Libionka W, Han X et al (2006) Astrocyte mediated control of cerebral blood flow. Nat Neurosci 9(2):260–267
Torii T, Miyamoto Y, Yamauchi J, Tanoue A (2014) Pelizaeus-Merzbacher disease: cellular pathogenesis and pharmacologic therapy. Pediatr Int 56(5):659–666
Tripathi R, Mc Tigue DM (2007) Prominent oligodendrocyte genesis along the border of spinal contusion lesions. Glia 55(7):698–711
Uranova NA, Vostrikov VM, Vikhreva OV, Zimina IS, Kolomeets NS, Orlovskaya DD (2007) The role of oligodendrocyte pathology in schizophrenia. J Neurooncol 10(4):537–545
Verkhratsky A, Nedergaard M (2018) Physiology of astroglia. Physiol Rev 98:239–389
Vikhreva OV, Rakhmanova VI, Orlovskaya DD, Uronovo NA (2016) Ultrastructural alteration of oligodendrocytes in prefrontal white matter in schizophrenia: a post-mortem morphometric study. Schizophr Res 177(1–3):28–36
Volterra A, Meldolesi J (2005) Astrocyte from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 6(8):626–640
Von Kolliker A (1919) The big-bang of modern glial biology: Translation and comments on del Río Hortega 1919 series of papers on microglia. Glia 64(11):1801–1840
Wang S, Sdrulla AD, di Sibio G, Bush G, Nofziger D, Hicks C et al (1998) Notch receptor activation inhibits oligodendrocyte differentiation. Neuron 21(1):63–75
Xu J, Chen S, Ahmed SH, Chen H, Ku G, Goldberg MP et al (2001) Amyloid- beta peptides are cytotoxic to oligodendrocytes. J Neurosci 21(1):118
Yin X, Crawford TO, Griffin JW, Tu PH, Lee VM, Li C et al (1988) Myelin associated glycoprotein is a myelin signal that modulates the caliber of myelinated axon. J Neurosci 18(6):1953–1962

Yu Z, Nagai J, Khakh BS (2020) Improved tools to study astrocytes. Nat Rev Neurosci 21(3): 121–138

Yuan X, Eisen AM, Mc Bain CJ, Gallo V (1998) A role of glutamate and its receptors in regulation of oligodendrocyte development in cerebral tissue slices. Development 125:2901–2914

Zhang Y, Barres BA (2013) A smarter mouse with human astrocytes. Bio Essay 35(10):876–880

Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T (2003) Neuron to astrocyte signalling is central to the dynamic control of brain microcirculation. Nat Neurosci 6(1):43–50

Basic Biology of Astrocytes

Pallavi Pant and Pankaj Seth

Abstract

Initially, glial cells were believed to function as "glue or packaging" cells of the brain; however, the last two decades of research from basic and clinical scientists have duly recognized them as one of the most important cell types in the mammalian brain. It has been now well established that glial cells, and, in particular, astrocytes, play immensely important roles that enable neurons to function optimally. This chapter describes the historical aspects of research on astrocytes, their role during brain development and synaptogenesis and other physiological functions of the brain, and finally how they contribute to disease pathogenesis or CNS disorders.

Keywords

Astrocytes · Blood-brain barrier · Glial cells · GFAP

1 Introduction

1.1 History

The documented history of the presence of cells apart from neurons in the nervous system dates back to nearly two centuries when Dutrochet, in 1824, identified two cell types in the mollusk brain and Gabriel Gustav Valentin, in 1836, proposed the idea of the existence of excitable active and non-excitable passive elements in the brain. In 1851, Heinrich Müller produced the first images of retinal radial glial cells,

P. Pant · P. Seth (✉)
Cellular and Molecular Neuroscience, National Brain Research Centre, Gurgaon, Haryana, India
e-mail: pseth.nbrc@gov.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_2

while Jacob Henle and Friedrich Merkel visualized the glial network in gray matter in the year 1869. Opposing the dominant opinion of the presence of only neurons in the brain in his times, Rudolf Ludwig Carl Virchow had an idea of the presence of another cell type in the brain which motivated him in their search (Parpura and Verkhratsky 2012). In 1856, he introduced the concept and the term "Nervenkitt" or "neuroglia"—as "connective tissue" supporting nervous elements that span from the ependyma to the deeper white matter (Somjen 1988). Using the absence of axon as a characteristic feature, first proposed by Deiters in 1865, Camillo Golgi provided the first detailed description of glial cells by observing thin sections fixed with osmic acid. He used silver nitrate chromate stain to characterize points of contact (now known as endfeet) between glial cells and blood vessels. In subsequent years, the idea of the origin of glial cells was shifted from mesodermal connective tissue to ectodermal-derived ependymal cells. In 1913, Santiago Ramón y Cajal identified astrocytes, their origin from radial cells, and the cell division property of adult astrocytes through gold and mercury sublimate staining of glial fibrillary acidic protein (GFAP). Later in 1920, del Río Hortega introduced the term microglia to describe non-neuronal "wandering histiocytes" in the central nervous system (CNS) with mesodermal origins and interfascicular glia with neuroepithelium origin called oligodendrocytes. The discovery of NG2 glia or oligodendrocyte progenitor cells came much later in 1981 by William Stallcup.

The shift of direction of research from morphological identification to the physiological functions of glial cells was gradual. With the development of electrophysiology, the study of neurons accelerated, while the glial cell research was left restricted to histochemical techniques. The evolution of glial cell function from the notion that it simply fills the empty spaces to its expansive and complex functions in pathological conditions took more than a hundred years. Cajal advanced the idea of glial cells and their processes to serve nerve fibers to provide nutrition for neurons (García-Marín et al. 2007). The idea of glia resembling secretory cells, initiated by Cajal, was further elaborated by Achucarro who proposed that the glial extensions secrete factors that communicate with the bloodstream. The phagocytic property of glial cells was first reported by Marinesco in 1896 which were later observed by del Río Hortega in microglia (Pérez-Cerdá et al. 2015). He identified most of the functions of microglia known in the present times. Beginning with the contribution of these eminent scientists, the scope of function of glial cells, especially astrocytes, is now expanded much more than just supporting cells in the brain.

1.2 Classification

In the mammalian nervous system, the glial cells are classified into peripheral nervous system (PNS) glia and central nervous system (CNS) glia. Thus, PNS glia are the glial cells present in the peripheral nervous system and include Schwann cells, olfactory ensheathing cells, and satellite glial cells. The Schwann cells are further divided into myelinating Schwann cells that myelinate axons of neurons in PNS, non-myelinating Schwann cells that surround PNS non-myelinated axons, and

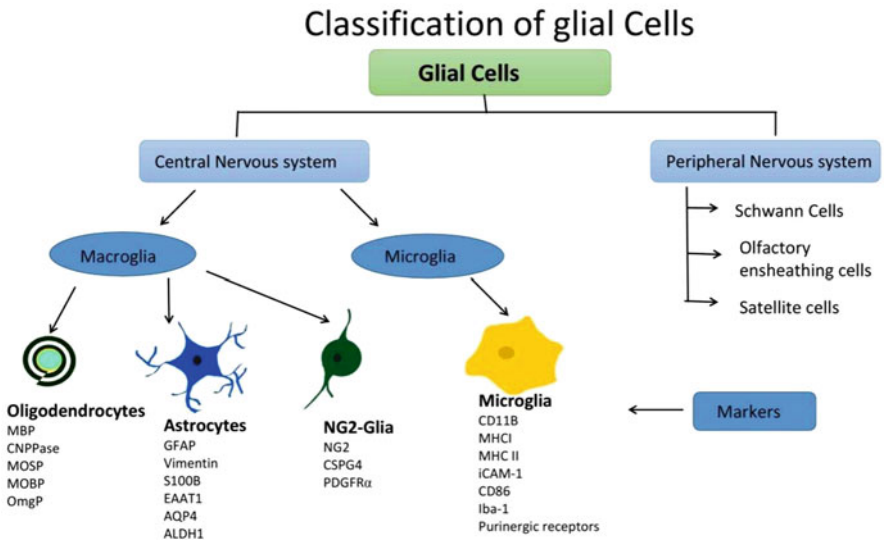

Fig. 1 Classification of glial cells and their cell-specific markers. The glial cells are present in CNS and PNS. The glial cells in PNS include Schwann cells, olfactory ensheathing cells, and satellite cells. The cells in CNS are grouped based on their ectodermal or mesodermal origins. The microglia have mesodermal origins and invade the brain parenchyma early in development. The macroglia have ectodermal origins and are classified into oligodendrocytes, astrocytes, and NG2 glia. Created on Inkscape Project, 2020. *Inkscape*, Available at https://inkscape.org

perisynaptic Schwann cells that wrap peripheral synapses. Satellite glial cells surround neurons in the peripheral ganglia. While the olfactory ensheathing cells are the radial glial cells that envelop unmyelinated olfactory axons, the enteric glial cells occupy the enteric nervous system. In the CNS, the glial cells are classified into two types based on their origins—microglia and macroglia—that include astrocytes, oligodendrocytes, and nerve/glial antigen 2 (NG2) glia. The microglia have a mesodermal origin and enter the brain early during embryogenesis, while the macroglia have ectodermal origins and are differentiated into the cell types based on the growth factors provided during the development. Microglia are the resident innate immune cells of the CNS that constantly scavenge the surroundings for possible pathogens and insults. As the name suggests, astrocytes are star-shaped cells with processes that interact with other glia cells, neurons, and the vascular endothelium to maintain blood-brain barrier integrity. Oligodendrocytes are responsible for generating myelin sheath around the axon throughout life, while NG2 glia cells are adult progenitor cells that tend to differentiate into astrocytes and oligodendrocytes. Each cell type has a specific function that contributes to the maintenance of homeostasis for synapses and proper circuit functioning (Fig. 1).

2 Embryogenesis and Development of CNS

2.1 Development of Glial Cells

The neurons and glia, except microglia, develop from the progenitor-mediated radial glial cells that line the ventricular cavities in the CNS of the embryo. The proliferative progenitor cells originating from the neuroepithelial cells give rise to neurons, astrocytes, or oligodendrocytes depending on the trophic factors provided in the environment. The glial fibrillary acidic protein (GFAP), astrocyte-specific glutamate transporter, brain lipid-binding protein, and RC2 antigen-expressing primate radial glia are differentiated into various glial cell types including NG2 glia, astrocytes, and oligodendrocytes at the embryonic day E10 in rats (Zhang 2001; Falk and Götz 2017). In adult neurogenesis, the neural stem cells (NSCs) that migrated to the subventricular zone (SVZ) during development commit to neuronal fate through astrocyte-secreted factors like Wnt7a and Ephrin-B2 and expression of Notch receptor ligands like Dlk1 (Falk and Götz 2017; Marchetti and Pluchino 2013; Kriegstein and Alvarez-Buylla 2009). Also, Wnt signaling activates the proliferation of OPCs into oligodendrocytes. The radial glia cells destined to become adult NSCs lose their proliferative property early in the developmental stage and express p^{57kip2} that shifts them to the quiescence stage of the cell cycle (Falk and Götz 2017). The radial glia polarity primarily supports the radial migration of neurons to form the cortex of the brain (Taverna et al. 2014). As the neurons migrate radially through bipolar radial cells, most of the radial cells lose their polarity and contact from ventricles and start transforming into astrocytes (Kriegstein and Alvarez-Buylla 2009). Astrocytes express adhesion molecules like Tenascin-C, Ephrin-B2, and Ephrin-B3 that help in the migration of olfactory interneurons and neuroblasts (Marín and Rubenstein 2003).

As neurons and glia regulate each other's number, the migration and differentiation of precursor cells are dependent on the growth factors activated and secreted through various signaling pathways (Taverna et al. 2014). The bone morphogenetic protein (BMP)-induced Smad signaling differentiate NSCs into astrocytes (Sun et al. 2001), while blocking fibroblast growth factor (FGF) signaling, the astrocytes change morphology to reactive astrocytosis by increasing the expression of GFAP (Farmer and Murai 2017). The Notch signaling inhibits neurogenin 1 (ngn1)-meditated neuronal differentiation (Farmer and Murai 2017) and specifies the fate of ectodermal cells by expressing delta proteins that bind to the notch present on the adjacent cell-activating notch signaling to continue with the glioblast lineage (Gaiano and Fishell 2002). Further, the gain of function of notch allows the transcription of *glial cells missing (gcm)* gene, which encodes a nuclear transient protein selectively expressed in early glial cells and acts as a switch to neuronal fate, if inhibited (Hosoya et al. 1995; Jones et al. 1995). Although Notch can promote Müller glial, radial glial, and astroglial fates in the mammalian brain, its activation inhibits oligodendroglia differentiation as well (Behzadian et al. 1998). Moreover, extrinsic growth factors like the cytokines leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) are potent inducers of astrocyte production via

the JaK/STAT pathway independent of notch potentiation of precursor cells (Sun et al. 2001). The immature astrocytes contact neurons and release factors that potentiate a neuron to form a synapse with the other neurons. Microglia along with oligodendrocytes play an essential role in the axon guidance (Allen and Lyons 2018; Meng et al. 2016). Also, astrocytes define circuits, synaptic pruning, and phagocytosis of failed synapses to eventually play a major role in synapse formation.

2.2 Synaptogenesis and Synapse Maintenance

Astrocytes play a dominant role in the development of the CNS through their involvement in the formation and maintenance of accurate synapses for a functional neural network across the brain. Astrocytes direct the neurons to their location, facilitate neurite growth, and influence the formation and strengthening of the synapse (Bialas and Stevens 2012; Kuijlaars et al. 2016; Stogsdill et al. 2017; Tsai et al. 2012; Ullian et al. 2004). The process of synaptogenesis starts in midgestation and continues extensively for 3 weeks post-natal in rats (8 weeks post-gestation to 2 years post-natal in the human cerebral cortex). This process allows the neurons to form elaborate yet precise connections with each other through enhanced efficacy of pre-synaptic transmitter release, redistributed receptors, strengthened contacts, and expression of neuron type-specific receptors (Bosworth and Allen 2017; He and Sun 2007; Slezak and Pfrieger 2003). Remarkably, the astrocytes are allocated to specific location regions of the brain during development to sustain synaptogenesis. They fail to provide appropriate surroundings for synaptogenesis when recruited from a different region (Tsai et al. 2012).

The function of glial cells, including astrocytes, in the development of a synapse can be categorized into the efficient processes of axon guidance, synaptogenesis, circuit redefining, and clearance of failed synapses. Firstly, they guide the neurons toward the target and promote neurite growth through the secretion of factors like thrombospondin, an extracellular protein (Mosser et al. 2017). The astrocytes, microglia, and oligo play an essential role in axon guidance and support the migration of the neurons in a calcium-dependent manner (Meng et al. 2016). The neurite growth is significantly reduced when astrocytes have low expression of N-cadherin along with reduced calcium signaling (Pfrieger 2009). Another protein that guides glia-mediated growth of axon is netrin. The netrin expression of the post-synaptic neuron directs the neuron toward the glia already in contact with the pre-synaptic neuron (Colón-Ramos et al. 2007). Microglia specifically affect the development of dopaminergic axons through brain-derived neurotrophic factor (BDNF)-mediated tropomyosin receptor kinase B (TrkB) signaling while leaving serotonergic axons unaffected (Mosser et al. 2017). Also, the astrocytes express neuroligin, an adhesion molecule that helps the neuron to achieve its ultimate aim to reach the target with a morphologically correct dendrite that is ready to form a connection with the other neurons.

Secondly, synaptogenesis is an extensive and meticulous process that requires the accurate and precise functioning of all the cell types present in the brain. The process of synaptogenesis coincides with gliogenesis wherein the immature astrocytes contact neurons and release factors that potentiate the neuron to form a synapse with other neurons (Allen 2019). Neurons form fewer and non-functional synapses without glial cells, while their presence increases not just the number of neurons but the efficiency of the synapses formed. Astrocyte-conditioned media (ACM) studies show the presence of apoE-containing lipoproteins as secretory factors in the media that increase the total number of synapses on each neuron and their maintenance by dendrite development along with neuronal survival (Mauch et al. 2001; Barres and Smith 2001). Thrombospondin is another factor identified in the ACM which is secreted by astrocytes that induces synapse formation (Allen and Barres 2005) Christopherson et al. 2005). Other confirmed soluble factors include Hevin, BDNF, transforming growth factor-α (TGF-α), insulin-like growth factor-1 (IGF-1), tumor necrosis factor-α (TNF-α), and secreted protein acidic and rich in cysteine (SPARC) (Baldwin and Eroglu 2017; O'Kusky et al. 2000; Stellwagen and Malenka 2006). The BDNF facilitates synaptogenesis through spine formation regulated by microglia which is observed in studies where BDNF was specifically knocked out (Mosser et al. 2017). TNF-α and thrombospondins increase synapse formation efficiency by increasing the expression of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the excitatory neurons while decreasing the expression of inhibitory receptors through promotion of endocytosis (Mosser et al. 2017; Freeman and Rowitch 2013). IGF-1 promotes synaptogenesis and facilitates myelination of the neurons that favors neuron survival and increases the number of synapses per neuron (D'Ercole et al. 2002; O'Kusky et al. 2000). Astrocytes regulate the excitatory synapses by enhancing their number with the release of glutamate receptor agonists and suppress them by adenosine 5′-triphosphate (ATP) release. The inhibitory neural networks are also influenced by the presence of astrocytes. Apart from secretion of soluble factors, glial cells also improve synaptogenesis through physical contacts (Allen and Barres 2005). The expression of adhesion molecules including Ephrin-A3 and Hevin by the glial cells allow their association with the dendritic spines to bridge the gap between pre-synaptic and post-synaptic neurons (Allen and Barres 2005; Baldwin and Eroglu 2017; Hama et al. 2004). Astrocytes also express neuroligin (NL) family protein NL2 that interacts with neurexins in neurons to allow the formation of excitatory synapse, while its downregulation results in inhibitory synapses (Stogsdill et al. 2017). On the contrary, they can physically prevent the neurons to contact each other and thereby prevent synaptogenesis.

Externally supplied cholesterol also sustains continuous synapse development and the stability of the evoked release of neurotransmitters. As the synapses between the neurons are developed with the support of glial cells, the circuits are refined in an activity-dependent manner. The "fine-tuning" is essentially required for the cancellation of unnecessary noise. With the presence of purinergic receptors on their surface, astrocytes and microglia are recruited to the activity-dependent ATP released by the neurons for their elimination (Miyamoto et al. 2013; Fatima et al.

2017). Microglia maintain contact with the synapse through spines, pre-synaptic terminal, and synaptic clefts that increases the neuronal activity to define an established circuit (Arcuri et al. 2017). Microglia help in redefining the circuits by removing inappropriate synapses in an activity-dependent manner (Mosser et al. 2017). Uptake of glutamate from the extracellular space by astrocytes through the expression of amino acid transporter by astrocytes suppress the synaptic strength, reported in drosophila (Grosjean et al. 2008). This suggests the possible role of these transporters during development that refines a circuit by elimination of glutamatergic synapses due to inactivity. Neurons rely on astrocytes to recycle neurotransmitters such as glutamate and histamine for sustained signaling. Perisynaptic astrocytes take in and convert neurotransmitters including glutamate, gamma-aminobutyric acid (GABA), and histamine into inactive metabolites for transport; therefore, astrocytes adjust synaptic communication and plasticity (Allen and Lyons 2018). The timing of synaptogenesis coincides with astrocyte generation. With the progression of synapse maturation, the immature synapse needs to be eliminated by astrocytes and microglia. Mice lacking CX3C chemokine receptor 1 (CX3CR1) have delayed synaptic pruning when microglia numbers are reduced. As a downstream target of CX3CR1, IGF-1 is secreted as a trophic factor by glial cells that promotes BDNF-mediated neuronal survival (O'Kusky et al. 2000). Also, astrocytes indirectly participate in the debris clearance process by secreting phagocytic markers that are recognized by microglia for phagocytosis (Bosworth and Allen 2017). Thus, astrocytes not only support but contribute significantly to the synaptogenesis and maintenance of the synapse during the development of the CNS.

3 Markers and Functions

3.1 Cell-Specific Markers

Astrocytes

Astrocytes are star-shaped glial cells that change their morphology into activated phenotypes in response to a threat. As the astrocytes develop, the immature astrocytes express an intermediate filament vimentin which is replaced by GFAP as they differentiate into their mature forms (Bramanti et al. 2010). GFAP is an approximately 9 nm intermediate filament cytoskeletal protein that provides structural stability and helps in the motility of the astrocytes. This cytoskeletal protein is largely expressed by mature astrocytes with increased expression following CNS injury, reactive astrogliosis, and glial scars (Zhang 2001; Zhou et al. 2020). The increased expression and accumulation of GFAP in the cytosol in response gliosis make it a characteristic marker for astrocyte reactivation. Another protein used for their identification is S100β, a constitutively expressed protein, primarily produced by astrocytes that exerts an autocrine and paracrine effect on the neural cell types to induce the expression of nitric oxide and pro-inflammatory cytokines. S100β is a calcium binding protein that induces the neurotrophic function of cell growth and proliferation in primary astrocytes (Schönrock et al. 1998). Therefore, elevated

levels of S100β in the serum or tissue correspond to reactive astrocyte state. As glutamate and its transporters are majorly responsible for maintaining the homeostasis in the synaptic cleft, the specific receptors on the membrane surface serve as biomarkers of the cell. This includes the glutamate transporter 1 (GLT1) or excitatory amino acid transporter 2 (EAAT2) which is predominantly localized in the astrocytes. The GLT1 transports one molecule of glutamate from the extracellular space with three Na^+ ions and an H^+ ion. As GLT1 is predominantly localized in the astrocytes, they are used as cell-specific markers. Other proteins used for astrocyte identification include aldehyde dehydrogenase 1 (ALDH1) and aquaporin 4 (AQ4). The change in expression levels of these biomarkers also helps in the identification of the state of astrocytes (Bramanti et al. 2010).

3.2 Functions

The glial cells have expanded from a mere two to three cells in numbers in *C. elegans* to consisting more than 90% of total cells in mammalian brain parenchyma. With evolution, not only the numbers increased, but their contribution in sustaining the proper functioning of the neurons and the brain also evolved. From their remarkable function in the development of CNS and synaptic transmission to the structural support and barrier function, glial cells have a diverse range of roles that ultimately maintain homeostasis in physiological conditions and try to restore it in pathological conditions. The microglia act as the resident immune cells that respond to the insults and pathogens, while astrocytes provide structural and functional support to the blood-brain barrier (BBB) and the neurons (Arcuri et al. 2017; Bagchi et al. 2019; Chen and Li 2021; Graeber and Streit 2010; Uanglei et al. 2010). NG2 glia are adult progenitor cells that can differentiate into both astrocytes or oligodendrocytes. NG2 cells have the tendency to generate and maintain oligodendrocytes which is essential for neuronal myelination for axonal support and fast conduction of signals. The functions of each class of glial cells are not restricted to the functions mentioned but diversify to wider range to support the neuronal survival and thus the homeostasis of the central nervous system.

The astrocytes play an essential role in instructing the fate of cells during the development of CNS along with operation in synaptogenesis by increasing the number of mature functional synapses. Although few studies show an important role of microglia in early synaptogenesis (when astrocytes are not present) during embryonic days 14–15 in rats, the majority have proved a dominant phagocytic role of microglia to clear out the dying neurons in later stages of embryonic development (Pont-Lezica et al. 2011). Astrocytes provide structural support and a physical barrier to the brain that allows the brain to be separated from the rest of the body systems and provide a blockade for substances' as well as pathogens' entry to the brain. The role is not limited to structural support but also the functional integrity of the blood-brain barrier through the formation of the neurovascular unit, where the endfeet of the astrocytes are in close association with the microvascular endothelium to allow selective passage of substances.

Astrocytes support metabolic needs, nutrition, and ion exchange to maintain the proper functioning of neuronal circuits. They provide lactate as a substrate to neurons in an activity-dependent manner, allow glutamate uptake through EAAT and control extracellular K+ to regulate water transport (Ransom and Sontheimer 1992). Glutamate, a neurotransmitter, is recovered from the synaptic cleft through glutamate receptors on the astrocyte and transferred as glutamine back to neurons (Lobsiger and Cleveland 2007). Another function of glial cells includes the regulation of neuronal survival during development through continuous neurotropic signals which helps in their proliferation and differentiation. While astrocytes aid in synaptogenesis, microglia continuously scavenge the environment to assist in axon fasciculation, neurite formation, and synaptic pruning and engulf the majority of cellular debris (Wake et al. 2012). The NG2 glia are adult progenitor cells that have the potential to differentiate into astrocytes and oligodendrocytes (Verkhratsky et al. 2019). When the brain parenchyma encounters insult, the astrocytes send neurotrophic signals for their migration, proliferation, and differentiation into either cell type to compensate for the lost population (Nakano et al. 2017). Due to this reason, the NG2 glia and oligodendrocytes have high metabolic turnover and exhibit damage in the majority of pathologies. On the contrary, the astrocytes and microglia change their state of active rest to a reactive state that secretes soluble factors including cytokines, chemokines, and growth factors to maintain the homeostasis for adequate neuronal functioning.

Role of Astrocytes in Blood-Brain Barrier (BBB)

The neurons function in a sensitive, controlled, and regulated system that allows precise signaling among the neural networks. Thus, it is possible that an evolutionary pressure was created to protect the brain from neurotoxins and isolate the CNS from the rest of the system for its better functioning. The blood-brain barrier aids it by providing a physical blockade and yet allows efficient communication, selective permeation of molecules, and restricted pathogen entry to maintain homeostasis of the CNS. The blood-brain barrier mainly comprises the endothelial cells of the blood vessels, the perivascular space, and the endfeet of astrocytes allowing the communication of the peripheral system to the brain. When astrocyte aggregates are cultured with endothelial cells, astrocytes induce the formation of tight capillaries and venules as opposed to when endothelial cells are placed with meningeal cells in the iris (Janzer and Raff 1987; Quintana 2017). In the case of pathogenic invasion, the disruption of the BBB is primarily important to invade and infect the brain parenchyma. Many viruses like HIV, Japanese encephalitis, rabies, and West Nile virus utilize different mechanisms to breach this barrier through either paracellular or transcellular pathways (Dallasta et al. 1999; Dewhurst et al. 1987; Diamond and Klein 2004; Roy and Hooper 2008; Serramía et al. 2015; Spindler and Hsu 2012; Verkhratsky et al. 2010). For example, the West Nile virus changes the expression of tight junction proteins on endothelial cells, while rabies enters the brain through the neuronal route causing inflammation to disrupt the BBB (Chen and Li 2021). The astrocytes and endothelial cells have a synergistic effect on each other where the astrocytes release factors including vasoactive agents and cytokines to modify the

expression of junction proteins like occludin and zonula occludens 1 (ZO-1) and permeability of the endothelial cells, while they enhance differentiation of astrocytes by secreting factors like LIF (Janzer and Raff 1987; Abbott et al. 2010; Quintana 2017). Moreover, astrocytes are the primary source of inhibitory factors which downregulate the growth of endothelial cells and drive them toward apoptosis (Behzadian et al. 1995). This signifies that pathogens that can infect astrocytes can also disrupt the BBB by downsizing the endothelial cells and decreased angiogenesis.

Role of Astrocytes in Brain Functions

The astrocytes play an essential role in the CNS due to their close communications with neurons, synapses, blood vessels, and other glial cells (He and Sun 2007; Jessen 2004; Pfrieger 2010). Astrocytes provide a diverse functional capability with intricate molecular and structural properties that involve mechanical support to the neurons, a close association with the vascular endothelial cells for BBB, and maintaining extracellular molecules and neurotransmitters to name a few. The importance of astrocytes is established from the beginning of embryonic development of the CNS when they play a crucial role in neurite growth and synaptogenesis. The neurite growth is significantly reduced when astrocytes have low expression of N-cadherin along with reduced calcium signaling (Pfrieger 2009). As synaptogenesis coincides with gliogenesis, the immature astrocytes contact neurons and release factors that potentiate the neuron to form a synapse with other neurons (Allen 2019). Neurons form fewer and non-functional synapses without glial cells, while their presence increases not just the number of neurons but the efficiency of the synapses formed. Thrombospondins in the glial-conditioned media (GCM) which is secreted by astrocytes induce synapse formation (Allen and Barres 2005; Christopherson et al. 2005). Other identified soluble factors include Hevin, BDNF, TGF-α, IGF-1, TNF-α, and SPARC (Baldwin and Eroglu 2017; Cheng et al. 2007; O'Kusky et al. 2000; Stellwagen and Malenka 2006). TNF-α and thrombospondins increase synapse formation efficiency by increasing the expression of AMPA and NMDA receptors in the excitatory neurons while decreasing the expression of inhibitory receptors by promoting their endocytosis (Mosser et al. 2017; Freeman and Rowitch 2013). With the presence of purinergic receptors on their surface, astrocytes are recruited to the activity-dependent ATP released by the neurons for its elimination (Miyamoto et al. 2013). Also, astrocytes indirectly participate in the debris clearance process by secreting phagocytic markers that are recognized by microglia, inviting them to the site for phagocytosis (Bosworth and Allen 2017). The astrocytes express class II major histocompatibility complex (MHC II) antigens and co-stimulatory molecules B7 and CD40 which contribute to their immune function in the CNS (Dong and Benveniste 2001). Although less intense, TNF-α- and IFN-γ-induced expression of MHC II in the astrocytes act as antigen-presenting cells that may activate $CD4^+$ T-cell proliferation in the presence of B7 and CD40 and T-cell anergy or apoptosis in their absence in mice models. When the brain parenchyma encounters insults, the astrocytes send neurotrophic signals for their migration, proliferation, and differentiation into either cell type to compensate for the lost

population (Nakano et al. 2017). The adult NSCs reside with astroglial cells in the adult neurogenic niches (Falk and Götz 2017; Wen et al. 2009). This indicates that astroglial cells in these niches have an important role to provide extrinsic factors to NSCs to maintain their character or induce adult neurogenesis. This includes a complex interplay between various factors like Ephrin-B2 and Wnt and signaling pathways of BMP, Wnt, and Notch pathways (Table 1).

Role of Astrocytes in Neuronal Functions and Communication

During the development of CNS, astrocytes play a crucial role in the functioning of neurons beginning from forming and maintaining a synapse between neurons to controlling the homeostasis for precise signal transduction by modulating the concentrations of various molecules in the extracellular milieu. Astrocytes are closely associated with synapses through perisynaptic processes and aid in the regulation of neurotransmitter availability in the synaptic cleft. The presence of receptors of neurotransmitters on the astrocytes helps in their internalization and recycling or degradation. Astrocytes regulate glutamate concentration by a meticulous glutamate uptake process via amino acid transporters. Astrocytes also control extracellular K^+ that helps in the regulation of water transport and neurotransmitter uptake (Ransom and Sontheimer 1992). Glutamate, a neurotransmitter, is recovered from the synaptic cleft through glutamate receptors on the astrocyte and transferred as glutamine back to neurons (Lobsiger and Cleveland 2007). The astrocytes direct the excitatory as well as inhibitory synapses among the neurons to regulate the neural networks. They regulate the excitatory synapses by enhancing the release of glutamate receptor agonists and suppress them by releasing ATP. Astrocytes also express neuroligin family protein NL2 that interacts with neurexins in neurons that allows the formation of excitatory synapse, while its downregulation results in inhibitory synapses (Stogsdill et al. 2017).

Another role of astrocytes in development is contributed by their ability to metabolize pregnenolone in progesterone by 3β-hydroxysteroid dehydrogenase (Garcia-Segura and Melcangi 2006). The steroid produced by the astrocytes acts as a neuromodulator for the development of neurons and its function by regulating the levels of ATP, mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinases (PI3K)/protein kinase B (Akt) pathways. Neurons are observed to form fewer synapses in the absence of astrocytes which otherwise release cholesterol bound to apolipoprotein E (apoE) which are then internalized by the neuron (Barres and Smith 2001). As apoE is primarily synthesized by astrocytes while its receptors are abundantly expressed by neurons, a strong glial-induced synapse promoting the function of astrocytes is observed.

The astrocytes not only help in synaptogenesis but also regulate behavior through their effect on the GABAergic neurons in the striatum. The striatal neurons release GABA which activates Gi-coupled GPCRs to induce calcium signaling in astrocytes (Nagai et al. 2020). This in turn cause an increased expression of thrombospondin-induced synapse formation through increased expression of AMPA and NMDA receptors (Freeman and Rowitch 2013). The behavioral outcome of Gi-coupled G-protein-coupled receptor (GPCR) activation in astrocytes by GABA released from

Table 1 The functions executed by astrocytes in the CNS and their functional components

Functions	Functional components
Development of CNS	Neurogenesis; cell migration and formation of gray matter layer; synaptogenesis, synapse maturation, and maintenance by thrombospondin; axonal guidance
Structural support	Formation of neurovascular unit—a structural barrier between blood vessels and brain parenchyma by the expression of tight junction proteins in endothelial cells which is regulated by the astroglial endfeet surrounding the vessels
Barrier function	Regulation of formation and permeability of blood-brain and CSF-brain barrier; formation of glial vascular interface
Homeostatic function	Control extracellular K+; pH regulation by Na+/H+ exchangers; water transport through AQP4; removal of neurotransmitters from extracellular space via glutamate, glycine, and GABA transporters
Metabolic function	Uptake of glucose; deposition of glycogen; provide lactate as energy substrate to neurons in an activity-dependent manner; acute effects of ATP and glutamate release on synaptic function; release neurotrophic factors such as nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3; glutamate receptor activation that triggers release of TNF-alpha
Synaptic transmission	Regulation of maintenance of synapse; providing glutamate for glutamatergic transmission; regulating synaptic plasticity; integrating synaptic fields; provide humoral regulation of neuronal networks through the secretion of neurotransmitters and neuromodulators; secrete factors like TNF-alpha, interleukins, and nitric oxide that control the long-term potentiation; secretion of neurotrophins to modulate remodeling, growth, and circuitry formation of neuronal networks; regulation of proliferation and differentiation of neural stem cells by decreasing expression of ephrin
Regulation of blood flow	Regulate local blood supply (functional hyperemia) through the secretion of vasoconstrictors or vasodilators like arachidonic acid and nitric oxide
Higher brain functions	Chemoception through—regulation of body Na+, homeostasis, regulation of CO2 and ventilatory behavior, sleep, memory and learning, hoxb8 deficit results in obsessive grooming behavior in mice
Brain defense, neuroprotection, and post-injury remodeling	Identification of insults to provide a protective response; increase in ATP concentration, post-lesion synaptic repair, and reactive synaptogenesis; brief synaptic contacts shift to prolonged contacts; glial scar formation at the site of injury to protect healthy tissue

neurons includes hyperactivity and disturbed attention. The neurons provide a constant extrinsic cue to astrocytes in the cerebellum through sonic hedgehog (Shh) signaling to modulate their transcriptional profile. This neuron-modulated Shh signaling provides a transcriptional diversity to astrocytes in the cerebellum, cortex, and hippocampus (Farmer and Murai 2017). The interaction between astrocytes and neurons is a dialogue in which the neurons maintain the gene expression in astrocytes.

4 General Pathophysiology of Astrocytes

The discovery of glial cells was made by Virchow while observing a pathological brain. The fundamental function of the glial cells is to maintain the homeostasis of the brain. The failure of functioning of any constituent of the CNS leads to a disequilibrium that causes cell death and ultimately neurological disorders. The malfunction of glial cells is detrimental to the CNS because neurons require glial cells to survive which is not a constrain for glial cell survival. In the case of insults, glial cells are recruited and activated to protect neurons and help in the recovery from the damage. The insult can be acute like trauma and stroke or chronic insults like neurodegeneration, demyelination, or metabolic disorders. The reasons for the homeostatic imbalance can range from the metabolic, molecular, or cellular levels to the organ level. At the molecular level, the astrocytes affect neurotransmission due to loss of glutamate receptor uptake, which disrupts calcium homeostasis and mitochondrial dysfunction. The energy dynamics of the neurons are disturbed when the glial cells lack sufficient energy stores to support themselves or the neighboring neurons. Moreover, the structural and functional support of the blood-brain barrier by the astrocytes is significantly disrupted causing a disturbance in its integrity. Although insults constitute the majority of the reasons for the failure of the glial cells to maintain homeostasis, cell senescence is an important element to consider in gliopathologies. When the astrocytes are triggered to a state of reactive astrogliosis, NG2 cells initiate gliogenesis; induction of oligodendrocytes to white matter death; and microglial activation. The transformation of neuroprotective function of astrocytes to neurotoxic function leads to detrimental effects in the CNS; and the extent of neuronal death and consequently the neurological deficits are proportional to the extent of the failure of glial cells to rebuild the homeostasis.

4.1 Reactive Astrogliosis

With more than half of the cell population, astrocytes are undeniably one of the first cell types to get affected by insults in the CNS. After the failure of its initial response of hypertrophy and proliferation, astrocytes are pushed to a state of reactive gliosis. The type of insults varies the phenotypic response of reactive astrogliosis, for example, ischemia results in the activation of neuroprotective genes, while pathogen-induced factors activate neurotoxic gene function to trigger a complex

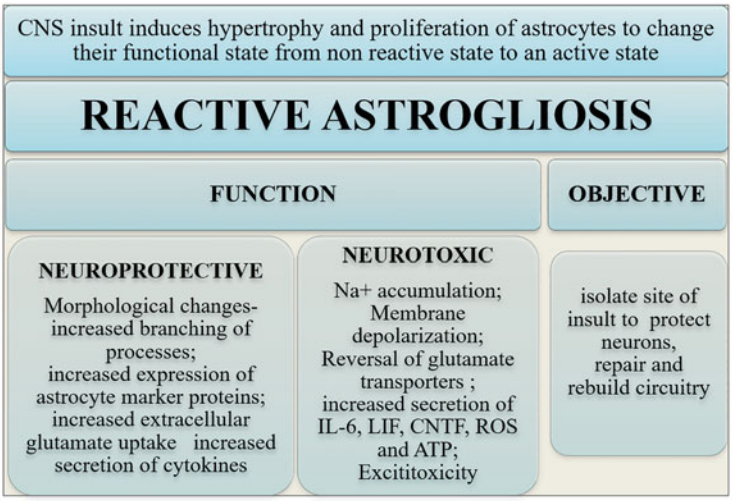

Fig. 2 Reactive astrogliosis is a neuroprotective mechanism of the astrocyte to isolate and repair the compromised site. The astrocytes transition into an active state when their increased migration and proliferation fail to counteract the insult

reaction irrespective of the etiology of the insult (Sofroniew 2009; Zamanian et al. 2012; Zhou et al. 2020). Histologically identified by the accumulation of glial fibers and glial scars, the morphological changes can be isomorphic or anisomorphic that progress gradually with time and severity of the perturbation. The main aim of reactive astrogliosis is to isolate the compromised area, protect the neurons, restore blood-brain barrier integrity, and rebuild the circuitry. While mild to moderate astrogliosis has the potential to regain a non-reactive state from hypertrophy and molecular and functional changes, severe astrogliosis renders the cells beyond repair and persists with scar formation and continuous inflammation. The active state of astrocytes is marked by the upregulation of GFAP, vimentin, S100β, ALDH1, and nestin along with the downregulation of GLT1/EAAT2 transporter proteins. Recent studies have identified Lipocalin-2 and Serpina3n as strong markers for astrogliosis with increased mRNA transcripts and protein expression (Zamanian et al. 2012) (Fig. 2).

Astrocytes are activated by the neurotoxic and pro-inflammatory factors released by activated microglia or damaged neurons. The extracellular secreted factors secreted by reactive astrocytes include cytokines like TGF-α, CNTF, IL-6, LIF, and IL-1, free radicals like nitric oxide and reactive oxygen species (ROS), and neurotransmitters like glutamate and ATP in response to an inflammatory stimulus (Mense et al. 2021; Pont-Lezica et al. 2011; Sharma et al. 2007). These factors induce physiological changes in the astrocytes that disturb membrane dynamics as well as cause metabolic dysfunction. Under pathological conditions, depolarization of astrocytes and Na^+ accumulation in the cytosol not only affect the potassium channels to increase the extracellular accumulation of K+ but also trigger a reversal

of glutamate transporter to increase glutamate in the extracellular milieu (Ransom and Sontheimer 1992). Moreover, when blood-brain barrier integrity is disrupted, the knockout of endothelial cell receptor cyclin-dependent kinase 5 (cdk5) downregulates the GLT1 through Cxcl1 chemokine leading to the further increase of glutamate in the extracellular space that in turn causes excitotoxic damage in the neurons (Liu et al. 2020). Consequently, epidermal growth factor receptor (EGFR) expression is presented in reactive astrogliosis which is usually absent in the non-reactive state of astrocytes. The histological condition of edema in glial scarring is a consequence of the upregulation of expression of plasma membrane integral protein, aquaporins, in the astrocytes that causes excess water outflow (Bramanti et al. 2010). Another membrane protein, connexin, which is a gap junction protein that physiologically connects astrocytes to other cells, can possibly be used for the passage of death signals that can initiate necrosis in the reactive astrocyte. The reactive astrogliosis upregulates various signaling pathways like cyclic adenosine monophosphate (cAMP), PI-3 K/Akt, JAK/STAT, and β-1 integrin-mediated signaling that increases the release of Ca^{2+} from the intracellular stores to cause mitochondrial as well as metabolic distress (Philips and Rothstein 2014). This dysfunction causes disorder and instability within the cell that ultimately forces the cell to undergo programmed cell death.

5 Conclusion

Glial cells in recent years attracted due attention of the basic as well as clinical neuroscientists on their roles in health and disease. Glial cells play several critical roles that help neurons in their genesis and guidance to appropriate location in the brain during development and in providing nourishment, maturation, and synaptogenesis. Much of the functions of glial cells are directed toward supporting neuronal functions and help them to function optimally. Recent discoveries of the role of astrocytes in physiology and pathophysiology of neurological disorders have further necessitated in-depth studies into their function in inflammation in both non-infectious and infectious diseases. The versatile functions of astrocytes in normal physiology justify their numbers as the most abundant cell type in the brain. While it is well established that astrocytes are critical for neuronal function, research efforts must be continued to understand the regulatory mechanisms that may govern the functions of glial cells. It is also necessary to develop good model systems of mixed brain culture of human origin and 3D cell culture models comprising the correct proportion of astrocytes and neurons along with oligodendrocytes and microglial cells, some of which have been achieved in brain organoids, but refinements are required. Furthermore, the role of astrocytes in the neuropathogenesis of emerging pathogens should be explored in detail for designing better therapeutic management.

References

Abbott NJ, Patabendige AAK, Dolman DEM, Yusof SR, Begley DJ (2010) Structure and function of the blood-brain barrier. Neurobiol Dis 37(1):13–25. https://doi.org/10.1016/j.nbd.2009.07.030

Allen NJ (2019) Star power: astrocytes regulate Behavior. Cell 177(5):1091–1093. https://doi.org/10.1016/j.cell.2019.04.042

Allen NJ, Barres BA (2005) Signaling between glia and neurons: focus on synaptic plasticity. Curr Opin Neurobiol 15(5):542–548. https://doi.org/10.1016/j.conb.2005.08.006

Allen NJ, Lyons DA (2018) System formation and function. Science 185:181–185

Arcuri C, Mecca C, Bianchi R, Giambanco I, Donato R (2017) The pathophysiological role of microglia in dynamic surveillance, phagocytosis and structural remodeling of the developing CNS. Front Mol Neurosci 10:1–22. https://doi.org/10.3389/fnmol.2017.00191

Bagchi S, Chhibber T, Lahooti B, Verma A, Borse V, Jayant RD (2019) In-vitro blood-brain barrier models for drug screening and permeation studies: an overview. Drug Des Devel Ther 13:3591–3605. https://doi.org/10.2147/DDDT.S218708

Baldwin KT, Eroglu C (2017) Molecular mechanisms of astrocyte-induced synaptogenesis. Curr Opin Neurobiol 45:113–120. https://doi.org/10.1016/j.conb.2017.05.006

Barres BA, Smith SJ (2001) Cation between nerve cells at meet-purified neurons to form synapse. Science 294:1296–1297

Behzadian MA, Wang X-L, Jiang B, Caldwell RB (1995) Angiostatic role or astrocytes: suppression of vascular endothelial cell growth by TGF-β and other inhibitory factor(s). Glia 15(4):480–490. https://doi.org/10.1002/glia.440150411

Behzadian MA, Wang XL, Shabrawey M, Caldwell RB (1998) Effects of hypoxia on glial cell expression of angiogenesis-regulating factors VEGF and TGF-β. Glia 24(2):216–225. https://doi.org/10.1002/(SICI)1098-1136(199810)24:2<216::AID-GLIA6>3.0.CO;2-1

Bialas AR, Stevens B (2012) Glia: regulating synaptogenesis from multiple directions. Curr Biol 22(19):R833–R835. https://doi.org/10.1016/j.cub.2012.08.036

Bosworth AP, Allen NJ (2017) The diverse actions of astrocytes during synaptic development. Curr Opin Neurobiol 47:38–43. https://doi.org/10.1016/j.conb.2017.08.017

Bramanti V, Tomassoni D, Avitabile M, Amenta F, Avola R (2010) Biomarkers of glial cell proliferation and differentiation in culture. Front Biosci 2:558–570. https://doi.org/10.2741/s85

Chen Z, Li G (2021) Immune response and blood–brain barrier dysfunction during viral neuroinvasion. Innate Immun 27(2):109–117. https://doi.org/10.1177/1753425920954281

Cheng A, Coksaygan T, Tang H, Khatri R, Balice-Gordon RJ, Rao MS, Mattson MP (2007) Truncated tyrosine kinase B brain-derived neurotrophic factor receptor directs cortical neural stem cells to a glial cell fate by a novel signaling mechanism. J Neurochem 100(6):1515–1530. https://doi.org/10.1111/j.1471-4159.2006.04337.x

Christopherson KS, Ullian EM, Stokes CCA, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA (2005) Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120(3):421–433. https://doi.org/10.1016/j.cell.2004.12.020

Colón-Ramos DA, Margeta MA, Shen K (2007) Glia promote local synaptogenesis through UNC-6 (netrin) signaling in C. elegans. Science 318(5847):103–106. https://doi.org/10.1126/science.1143762

D'Ercole JA, Ye P, O'Kusky JR (2002) Mutant mouse models of insulin-like growth factor actions in the central nervous system. Neuropeptides 36(2–3):209–220. https://doi.org/10.1054/npep.2002.0893

Dallasta LM, Pisarov LA, Esplen JE, Werley JV, Moses AV, Nelson JA, Achim CL (1999) Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. Am J Pathol 155(6):1915–1927. https://doi.org/10.1016/S0002-9440(10)65511-3

Dewhurst S, Sakai K, Bresser J, Stevenson M, Evinger-Hodges MJ, Volsky DJ (1987) Persistent productive infection of human glial cells by human immunodeficiency virus (HIV) and by

infectious molecular clones of HIV. J Virol 61(12):3774–3782. https://doi.org/10.1128/jvi.61.12.3774-3782.1987
Diamond MS, Klein RS (2004) West Nile virus: crossing the blood-brain barrier. Nat Med 10(12):1294–1295. https://doi.org/10.1038/nm1204-1294
Dong Y, Benveniste EN (2001) Immune function of astrocytes. Glia 36(2):180–190. https://doi.org/10.1002/glia.1107
Falk S, Götz M (2017) Glial control of neurogenesis. Curr Opin Neurobiol 47:188–195. https://doi.org/10.1016/j.conb.2017.10.025
Farmer WT, Murai K (2017) Resolving astrocyte heterogeneity in the CNS. Front Cell Neurosci 11:1–7. https://doi.org/10.3389/fncel.2017.00300
Fatima M, Prajapati B, Saleem K, Kumari R, Singal MS, C., & Seth, P. (2017) Novel insights into role of miR-320a-VDAC1 axis in astrocyte-mediated neuronal damage in neuroAIDS. Glia 65(2):250–263. https://doi.org/10.1002/glia.23089
Freeman MR, Rowitch DH (2013) Evolving concepts of gliogenesis: a look way back and ahead to the next 25 years. Neuron 80(3):613–623. https://doi.org/10.1016/j.neuron.2013.10.034
Gaiano N, Fishell G (2002) The role of notch in promoting glial and neural stem cell fates. Annu Rev Neurosci 25:471–490. https://doi.org/10.1146/annurev.neuro.25.030702.130823
García-Marín V, García-López P, Freire M (2007) Cajal's contributions to glia research. Trends Neurosci 30(9):479–487. https://doi.org/10.1016/j.tins.2007.06.008
Garcia-Segura LM, Melcangi RC (2006) Steroids and glial cell function. Glia 54(6):485–498. https://doi.org/10.1002/glia.20404
Graeber MB, Streit WJ (2010) Microglia: biology and pathology. Acta Neuropathol 119(1):89–105. https://doi.org/10.1007/s00401-009-0622-0
Grosjean Y, Grillet M, Augustin H, Ferveur JF, Featherstone DE (2008) A glial amino-acid transporter controls synapse strength and courtship in drosophila. Nat Neurosci 11(1):54–61. https://doi.org/10.1038/nn2019
Hama H, Hara C, Yamaguchi K, Miyawaki A (2004) PKC signaling mediates global enhancement of excitatory synaptogenesis in neurons triggered by local contact with astrocytes. Neuron 41(3):405–415. https://doi.org/10.1016/S0896-6273(04)00007-8
He F, Sun YE (2007) Glial cells more than support cells? Int J Biochem Cell Biol 39(4):661–665. https://doi.org/10.1016/j.biocel.2006.10.022
Hosoya T, Takizawa K, Nitta K, Hotta Y (1995) Glial cells missing: a binary switch between neuronal and glial determination in drosophila. Cell 82(6):1025–1036. https://doi.org/10.1016/0092-8674(95)90281-3
Janzer RC, Raff MC (1987) Astrocytes induce blood-brain barrier properties in endothelial cells. Nature 325(6101):253–257. https://doi.org/10.1038/325253a0
Jessen KR (2004) Glial cells. Int J Biochem Cell Biol 36(10):1861–1867. https://doi.org/10.1016/j.biocel.2004.02.023
Jones BW, Fetter RD, Tear G, Goodman CS (1995) Glial cells missing: a genetic switch that controls glial versus neuronal fate. Cell 82(6):1013–1023. https://doi.org/10.1016/0092-8674(95)90280-5
Kriegstein A, Alvarez-Buylla A (2009) The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32:149–184
Kuijlaars J, Oyelami T, Diels A, Rohrbacher J, Versweyveld S, Meneghello G, Tueffer M, Verstraelen P, Detrez JR, Verschuuren M, De Vos WH, Meert T, Peeters PJ (2016) Sustained synchronized neuronal network activity in a human astrocyte co-culture system. Nature 2020:1–14. https://doi.org/10.1038/srep36529
Liu X-X, Yang L, Shao L-X, He Y, Wu G, Bao Y-H, Lu N-N, Gong D-M, Lu Y-P, Cui T-T, Sun N-H, Chen D-Y, Shi W-X, Fukunaga K, Chen H-S, Chen Z, Han F, Lu Y-M (2020) Endothelial Cdk5 deficit leads to the development of spontaneous epilepsy through CXCL1/CXCR2-mediated reactive astrogliosis. J Exp Med 217:1. https://doi.org/10.1084/jem.20180992
Lobsiger CS, Cleveland DW (2007) Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. Nat Neurosci 10(11):1355–1360. https://doi.org/10.1038/nn1988

Marchetti B, Pluchino S (2013) Wnt your brain be inflamed? Yes, it Wnt! Trends Mol Med 19(3): 144–156. https://doi.org/10.1016/j.molmed.2012.12.001

Marín O, Rubenstein JLR (2003) Cell migration in the forebrain. Annu Rev Neurosci 26:441–483. https://doi.org/10.1146/annurev.neuro.26.041002.131058

Mauch DH, Na K, Schumacher S (2001) CNS synaptogenesis promoted by glia-derived cholesterol. Science 294:1354–1357

Meng L, Zhang A, Jin Y, Yan D (2016) Regulation of neuronal axon specification by glia-neuron gap junctions in C. elegans. Elife 5:e19510. https://doi.org/10.7554/eLife.19510

Mense SM, Sengupta A, Zhou M, Lan C, Bentsman G, Volsky DJ, Zhang L, Volsky DJ, Gene LZ (2021) Gene expression profiling reveals the profound upregulation of hypoxia-responsive genes in primary human astrocytes. Physiol Genomics 69:435–449. https://doi.org/10.1152/physiolgenomics.00315.2005

Miyamoto A, Wake H, Moorhouse AJ, Nabekura J (2013) Microglia and synapse interactions: fine tuning neural circuits and candidate molecules. Front Cell Neurosci 7:1–6. https://doi.org/10.3389/fncel.2013.00070

Mosser CA, Baptista S, Arnoux I, Audinat E (2017) Microglia in CNS development: shaping the brain for the future. Prog Neurobiol 149–150:1–20. https://doi.org/10.1016/j.pneurobio.2017.01.002

Nagai J, Rajbhandari AK, Gangwani MR, Hachisuka A, Masmanidis SC, Fanselow MS, Khakh BS, Sciences B, Behavior H (2020) Hyperactivity with disrupted attention by activation of an astrocyte synaptogenic cue. HHS Public Access 177(5):1280–1292. https://doi.org/10.1016/j.cell.2019.03.019.Hyperactivity

Nakano M, Tamura Y, Yamato M, Kume S, Eguchi A, Takata K, Watanabe Y, Kataoka Y (2017) NG2 glial cells regulate neuroimmunological responses to maintain neuronal function and survival. Sci Rep 7:6–7. https://doi.org/10.1038/srep42041

O'Kusky JR, Ye P, D'Ercole AJ (2000) Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. J Neurosci 20(22):8435–8442. https://doi.org/10.1523/jneurosci.20-22-08435.2000

Parpura V, Verkhratsky A (2012) Neuroglia at the crossroads of homoeostasis, metabolism and signalling: evolution of the concept. ASN Neuro 4(4):201–205. https://doi.org/10.1042/AN20120019

Pérez-Cerdá F, Sánchez-Gómez MV, Matute C (2015) Pío del Río hortega and the discovery of the oligodendrocytes. Front Neuroanat 9(July):7–12. https://doi.org/10.3389/fnana.2015.00092

Pfrieger FW (2009) Roles of glial cells in synapse development. Cell Mol Life Sci 66(13): 2037–2047. https://doi.org/10.1007/s00018-009-0005-7

Pfrieger FW (2010) Role of glial cells in the formation and maintenance of synapses. Brain Res Rev 63(1–2):39–46. https://doi.org/10.1016/j.brainresrev.2009.11.002

Philips T, Rothstein JD (2014) Glial cells in amyotrophic lateral sclerosis. Exp Neurol 262:111–120. https://doi.org/10.1016/j.expneurol.2014.05.015

Pont-Lezica L, Béchade C, Belarif-Cantaut Y, Pascual O, Bessis A (2011) Physiological roles of microglia during development. J Neurochem 119(5):901–908. https://doi.org/10.1111/j.1471-4159.2011.07504.x

Quintana FJ (2017) Astrocytes to the rescue! Glia limitans astrocytic endfeet control CNS inflammation. J Clin Investig 127(8):2897–2899. https://doi.org/10.1172/JCI95769

Ransom BR, Sontheimer H (1992) The neurophysiology of glial cells. J Clin Neurophysiol 9(2): 224–251

Roy A, Hooper DC (2008) Immune evasion by rabies viruses through the maintenance of blood-brain barrier integrity. J Neurovirol 14(5):401–411. https://doi.org/10.1080/13550280802235924

Schönrock LM, Kuhlmann T, Adler S, Bitsch A, Brück W (1998) Identification of glial cell proliferation in early multiple sclerosis lesions. Neuropathol Appl Neurobiol 24(4):320–330. https://doi.org/10.1046/j.1365-2990.1998.00131.x

Serramía MJ, Muñoz-Fernández MÁ, Álvarez S (2015) HIV-1 increases TLR responses in human primary astrocytes. Sci Rep 5:1–15. https://doi.org/10.1038/srep17887

Sharma V, Mishra M, Ghosh S, Tewari R, Basu A, Seth P, Sen E (2007) Modulation of interleukin-1β mediated inflammatory response in human astrocytes by flavonoids: implications in neuroprotection. Brain Res Bull 73(1–3):55–63. https://doi.org/10.1016/j.brainresbull.2007.01.016

Slezak M, Pfrieger FW (2003) New roles for astrocytes: regulation of CNS synaptogenesis. Trends Neurosci 26(10):531–535. https://doi.org/10.1016/j.tins.2003.08.005

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32(12):638–647. https://doi.org/10.1016/j.tins.2009.08.002

Somjen GG (1988) Nervenkitt: notes on the history of the concept of neuroglia. Glia 1(1):2–9. https://doi.org/10.1002/glia.440010103

Spindler KR, Hsu TH (2012) Viral disruption of the blood-brain barrier. Trends Microbiol 20(6):282–290. https://doi.org/10.1016/j.tim.2012.03.009

Stellwagen D, Malenka RC (2006) Synaptic scaling mediated by glial TNF-α. Nature 440(7087):1054–1059. https://doi.org/10.1038/nature04671

Stogsdill JA, Ramirez J, Liu D, Kim YH, Baldwin KT, Enustun E, Ejikeme T, Ji RR, Eroglu C (2017) Astrocytic neuroligins control astrocyte morphogenesis and synaptogenesis. Nature 551(7679):192–197. https://doi.org/10.1038/nature24638

Sun Y, Nadal-Vicens M, Misono S, Lin MZ, Zubiaga A, Hua X, Fan G, Greenberg ME (2001) Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms. Cell 104(3):365–376. https://doi.org/10.1016/S0092-8674(01)00224-0

Taverna E, Götz M, Huttner WB (2014) The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. Annu Rev Cell Dev Biol 30:465–502. https://doi.org/10.1146/annurev-cellbio-101011-155801

Tsai HH, Li H, Fuentealba LC, Molofsky AV, Taveira-Marques R, Zhuang H, Tenney A, Murnen AT, Fancy SPJ, Merkle F, Kessaris N, Alvarez-Buylla A, Richardson WD, Rowitch DH (2012) Regional astrocyte allocation regulates CNS synaptogenesis and repair. Science 337(6092):358–362. https://doi.org/10.1126/science.1222381

Uanglei GLI, Imon MEJS, Ancel LIMC, Hi ZHONGS, Inying XJI, Arbell JOHNMT, Iii BAMO, Ingmei BMFU (2010) Permeability of endothelial and astrocyte Cocultures. In Vitro Blood–Brain Barrier Model Drug Deliv Stud 38(8):2499–2511. https://doi.org/10.1007/s10439-010-0023-5

Ullian EM, Christopherson KS, Barres BA (2004) Role for glia in synaptogenesis. Glia 47(3):209–216. https://doi.org/10.1002/glia.20082

Verkhratsky A, Ho MS, Zorec R, Parpura V (2019) The concept of neuroglia. Adv Exp Med Biol 1175:1–13. https://doi.org/10.1007/978-981-13-9913-8_1

Verkhratsky A, Olabarria M, Noristani HN, Yeh C, Rodriguez JJ (2010) Astrocytes in Alzheimer's disease. Neurotherapeutics 7(4):399–412

Wake H, Moorhouse AJ, Nabekura J (2012) Functions of microglia in the central nervous system-beyond the immune response. Neuron Glia Biol 7(1):47–53. https://doi.org/10.1017/S1740925X12000063

Wen S, Li H, Liu J (2009) Dynamic signaling for neural stem cell fate determination. Cell Adh Migr 3(1):107–117. https://doi.org/10.4161/cam.3.1.7602

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32(18):6391–6410. https://doi.org/10.1523/JNEUROSCI.6221-11.2012

Zhang SC (2001) Defining glial cells during CNS development. Nat Rev Neurosci 2(11):840–843. https://doi.org/10.1038/35097593

Zhou Y, Shao A, Yao Y, Tu S, Deng Y, Zhang J (2020) Dual roles of astrocytes in plasticity and reconstruction after traumatic brain injury. Cell Commun Signal 18(1):1–16. https://doi.org/10.1186/s12964-020-00549-2

Oligodendrocyte: Structure, Function and Pathology

Nisha Patro, Ishan Patro, and P. N. Tandon

Abstract

Oligodendrocytes (OLs) are the myelinating cells of the central nervous system (CNS) and myelinate the axons facilitating and boosting the propagation and speed of nerve conduction. In addition, the OLs also provide metabolic support to neurons and enhance their viability, regulate ion and water homeostasis and play a crucial role in learning and memory via white matter (WM) plasticity. Loss of myelin/demyelination is common in many demyelinating and neurodegenerative disorders. The myelin loss may occur either due to direct damage to myelin sheath or indirectly by disruption or death of OLs due to autoimmune attack, injury, stroke and toxic insult or genetic defects involving intrinsic abnormalities in the production and maintenance of myelin. Loss of OLs usually triggers a regenerative remyelination causing the differentiation of OPCs into myelinating OLs and restoration of the myelin sheath. However, the regeneration is poor in many demyelinating disorders. Thus, enhancing remyelination strategies is of human health importance and is under active research.

Keywords

Oligodendrocytes · Myelination · White matter · Demyelination · Leukodystrophies

N. Patro (✉) · I. Patro
School of Studies in Neuroscience, Jiwaji University, Gwalior, India

P. N. Tandon
National Brain Research Centre, Manesar, Haryana, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_3

1 Oligodendrocytes

Oligodendrocytes (OLs), the myelinating cells of the CNS, are found in all vertebrates and are fundamental to myelin formation during development and critical for myelin regeneration following injury and demyelinating diseases. Myelin provides an evolutionary advantage in vertebrates by increasing axial resistance of the axonal surface in addition to reducing its capacitance (Castelfranco and Hartline 2015) and contributes to the emergence of complex and plastic behaviours (Tomassy et al. 2016). Oligodendrocytes are generated from neuroepithelial cells (NEPs) of the neural tube through a well-concerted process of migration, proliferation and differentiation (Davis and Temple 1994; Rogister et al. 1999; Bradl and Lassmann 2010). The fate specification of the NEPs to oligodendrocyte precursor cells (OPCs) is specifically regulated by the gradients of sonic hedgehog protein during early embryogenesis (Orentas et al. 1999; Cai et al. 2005). A number of transcription factors and epigenetic regulators, microRNAs and intracellular signalling pathways are known to drive the lineage progression of OLs (Emery and Lu 2015; Galloway and Moore 2016; Gaesser and Fyffe-Maricich 2016). Recent advances suggest the role of neuronal activity in the origin, proliferation and differentiation of OPCs and myelin remodelling (Barres and Raff 1993; Hughes et al. 2018). In the developing CNS, not all the OPCs that are generated get differentiated into mature myelinating glia; some undergo apoptosis as they fail to contact an appropriate axon, while others form a significant pool of adult OPCs (Tongatta and Miller 2016). OPCs represent as highly proliferative and migratory bipolar cell population, evenly distributed in WM and grey matter, although less abundant in grey matter (Dawson et al. 2003). In the developing CNS, an appreciable number of WM OPCs differentiate into myelinating OLs as compared to OPCs in the grey matter which persist as $NG2^+$ progenitors (Dimou et al. 2008; Kang et al. 2010). The $NG2^+$ progenitors express chondroitin sulphate proteoglycans, are highly dynamic and proliferative in adult CNS and maintain their population by self-renewal, differentiation and self-repulsion (Dimou et al. 2008; Kang et al. 2010; Hughes et al. 2013). The OPCs, which remain undifferentiated, are stored as potential backup progenitor pool and comprise majority of the proliferating cells in the adult CNS (Dawson et al. 2003). The proliferation and survival of OPCs are mediated by platelet-derived growth factor-α (PDGF-α) produced by astrocytes and neurons, via receptor for PDGF-α (PDGFR-α) expressed by OPCs; thus, PDGFR-α act as the best characterized marker for them (Noble et al. 1988; Calver et al. 1998). OPCs lose their bipolarity, differentiate into pre-OLs by expressing myelin-specific 2′-3′-cyclic-nucleotide 3′-phosphodiesterase (CNPase) and the cell surface (O4 and O1) markers (Sommer and Schachner 1981; Braun et al. 1988) and start contacting the target axon to myelinate. Further differentiation into the mature oligodendrocytes is associated with the production of myelin and expression of myelin proteins, myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), galactocerebroside (GalC) and myelin-oligodendrocyte glycoprotein (MOG) (Kuhn et al. 2019). A number of intracellular and extracellular signalling molecules are involved in maintaining the balance between OPC proliferation and differentiation (Hughes et al. 2013).

1.1 Myelination

Myelin is a multilamellar lipid structure that wraps around the axons, enables efficient saltatory conduction of nerve impulses and reduces axonal energy consumption (Huxley and Stampfli 1949). In addition to myelination and facilitating propagation and speed of nerve conduction, OLs also provide metabolic support to neurons to enhance their viability, regulate ion and water homeostasis and thus play a crucial role in learning and memory via WM plasticity and activity-dependent adaptive responses in myelin-forming cells that affect the formation of neural circuitry (Funfschilling et al. 2012; Lee et al. 2012; Philips and Rothstein 2017; Monje 2018; Stadelmann et al. 2019).

Pre-oligodendrocytes, the mitotically active OPCs, are the most prominent cell population during 18–28 weeks of human gestation. These cells subsequently become post-mitotic and switch to immature oligodendrocytes, start contacting axons and initiate myelination during 28–40 weeks of gestation (Craig et al. 2003; Dean et al. 2011). Thus, the gestation period between 23 and 32 weeks is considered to be the critical period of oligodendroglial maturation because it is when the OLs are highly vulnerable to infections, hypoxic-ischaemic injury and other insults (Hagberg et al. 2002; Semple et al. 2013). WM is especially more susceptible than grey matter in the pre-term and term infants and also in children, with infections and hypoxic-ischaemic injury or both in conjunction as the most common cause of WM lesions (Hagberg et al. 2002). The myelination in rodents occurs between postnatal days (PND) 10 and 14 and peaks around PND20, when most of the mature OLs expressing specific markers required for myelination and paranodal loop formation are frequently seen (Wiggins et al. 1986; Cahoy et al. 2008). The myelination in mice is completed in first two postnatal months, and the myelination of specific brain areas well correlates with the development of the cognitive functions and clearly depends on the brain area, time course of life, type of neurons, axonal diameter and environmental milieu (Tomassy et al. 2016). Moreover, the myelinated axons also differ in the number, distribution, internodal length and thickness of myelin sheath that defines the precise conduction times and nervous system plasticity (Fields 2015; Klingseisen and Lyons 2018). Development and differentiation of OPCs into myelinating oligodendrocytes can occur independent of axons (Almeida and Lyons 2016) as reported by in vitro studies where OLs can differentiate, mature and efficiently extend processes independent of neurons, suggesting their default potential for differentiation, and launch a programme of myelin gene expression (Simons et al. 2000; Klingseisen and Lyons 2018).

Myelination during development occurs in a conserved and region-specific pattern and follows a complex spatiotemporal sequence depending on the position of the system in the functional hierarchy, initiated in areas dedicated to basic homeostasis, progressing to areas involved in more complex tasks and finally in the areas required for higher-order functions (Yakovlev and Lecours 1967; Brody et al. 1987; Kinney et al. 1988). Moreover, the peripheral nervous system, brainstem and spinal cord are myelinated earlier than the brain, generally advancing from inferior to superior and caudal to rostral (Inder and Huppi 2000). Regions of the occipital

lobe are myelinated first and then the temporal and followed by the frontal lobe (Brody et al. 1987; Tasker 2006; Volpe 2000). More so, myelination is faster and finished early in regions that are myelinated first. Early researchers believed that myelination is complete by 3–5 years in humans along with many of the major tracts well myelinated by early childhood (Dietrich et al. 1988; Nakagawa et al. 1998). But more recently it has been reported that axons continue to be myelinated by the second to third decades of the human life, contributing to a net linear increase in the total WM volume by 12% between the ages of 4 and 22 years (Giedd et al. 1999). Thus, the pattern of myelination in the nervous system is a complex process and requires a continuous signalling between the axons and the OLs to select the axons and part of axons that are to be myelinated. The factors mediating such signalling are not well elucidated. In fact, the OLs in culture can myelinate paraformaldehyde-fixed axons (Rosenberg et al. 2008) and even inert axon-shaped fibres (Mei et al. 2016). Moreover, the sheath-like structure covering the inert axon-shaped fibres resembled that of compact myelin, but the inert fibres only with a diameter of 0.4 μm and more were myelinated in vitro, similar to an in vitro observation (Remahl and Hideberg 1982; Lee et al. 2012; Bechler et al. 2015). Using the inert axon-like fibre model, Bechler and group (2015) further reported that when oligodendrocytes were cultured on inert fibres of mixed diameter from 0.4 to 4.0 μm, the myelin sheath was more and even longer around larger diameter fibres, again similar to an in vivo observation made more than a century back by Donaldson and Hoke in 1905. These studies suggested that the length of the myelin sheath may be regulated by the axon and its diameter (Donaldson and Hoke 1905). However, the mechanisms by which the axons differentiate into the fibres of varying diameters and regulate the myelin length and thickness just by contact remain a matter of speculation. Moreover, the dendrites are never myelinated, indicating that diameter alone may not be responsible for the selection of axons by oligodendrocytes for myelination. The complete myelination requires electrically active neurons and healthy axons. It is now clear that extrinsic signals are required for the precise selection of axons for myelination as the oligodendrocytes show the bias only for axons, avoid inappropriate targets and show preference for more active axons (Wake et al. 2015; Koudelka et al. 2016). Once the internodal myelin sheath is formed, it grows both radially and longitudinally to a specific thickness and length that provide specific conduction property to the axon (Ford et al. 2015). The role of neuregulin-ErbB signalling is primarily important for the myelination and identification of axon calibre in the peripheral nervous system by Schwann cells (Birchmeier and Nave 2008), while its role is insignificant in the CNS myelination (Brinkmann et al. 2008). Moreover, Schwann cells are not able to myelinate the inert fibres (Bechler et al. 2015). Although the role of electrical signalling, glutamate release and neuregulin signalling or interactions with extracellular matrix molecules have been reported to modulate myelination in a limited way (reviewed by Stadelmann et al. 2019), further research is required to explore in more detail the factors that would correlate the signals with the axon calibre to determine myelination.

Myelination is developmentally prolonged in humans than in non-human primates. In chimpanzees, density of myelinated axons reaches to the maximum

level by adolescence in most cortical areas. However, in the human cerebral cortex, only a few numbers of axons are myelinated by birth; the myelination is slower during childhood and extended beyond adolescence to early adulthood. Moreover, the primary cortical areas are myelinated earlier than the association cortical areas followed by the late maturing brain areas such as pre-frontal cortex in the last (Yakovlev and Lecours 1967; Knickmeyer et al. 2010; Shaw et al. 2008; Miller et al. 2012). Most of the association areas continue to be myelinated in the third decade of life (Lebel et al. 2012; Williamson and Lyons 2018). The dynamic maturation of association and projection pathways involved in maintaining cortical and brainstem integration occurs during adolescence and is accompanied by the maturation of critical cognitive functions (Asato et al. 2010; Kumar et al. 2013). Thus, the gradual maturation of WM from childhood to early adulthood is fast and dynamic with remarkable increase in fibre density and the myelination (Lebel et al. 2019). WM structure becomes more or less static by mid-adulthood and subsequently shows degenerative changes during the latter part of life (Lebel et al. 2012).

The development of an individual is associated with the maturation of WM pathways that connect distant and proximal brain regions and are essential elements of higher-order cognitive processing (Fields 2010; Buyanova and Arsalidau 2021). About more than 50% of the adult brain volume represents WM and is crucial for sensory (Chang et al. 2016), motor (Hollund et al. 2017) and higher-order executive functions (Ohlhauser et al. 2018). Communication of neural signals is essentially required for humans to move, think, feel and respond. WM consists of neuronal fibres with varying degrees of myelination that allow the transfer of signals across different brain regions at different rates. Moreover, most glia are generated during either late embryogenesis or postnatal life, suggesting that development continues beyond birth and even adolescence. With glia being the dynamic cells, the continued active neuron-glia interactions actively chisel and remodel the nervous system throughout life.

1.2 Structure and Composition of Myelin

Myelin is a large spade-like extension of the plasma membrane of the myelinating glia that wraps around the axons to form a multi-layered stack visualized as a periodic structure of alternating major dense and intraperiod lines at the tightly apposed and compacted cytoplasmic and outer membrane surfaces, respectively, resulting in the periodicity of ~12 nm (Aggarwal et al. 2011; Nave and Werner 2014). The compacted myelin is devoid of cytoplasmic components except at the edges, where it forms a continuous network of clustered cytoplasmic channels and forms complex axo-glial junctions between the terminal ends of the myelin sheath, ~4 μm long, called as paranodal loops and 10–15-μm-long inner tongue that runs along the axon under the myelin sheath called as juxtaparanode (Hildebrand et al. 1993; Nave 2010; Stadelmann et al. 2019). In between the paranodes of the two adjacent myelin sheaths is the nodal region which is covered by paranodal astrocytic processes. The axon is usually constricted in the nodal region and is strikingly visible

in larger fibres (Hildebrand 1971). The paranodal axon-glial junctions provide electrical insulation by restricting current flow beneath the myelin sheath and help to segregate the voltage-gated sodium channels at the nodes from the potassium channels at the juxtaparanodes (Rasband et al. 2001).

Three integral myelin proteins, myelin basic protein (MBP), 2′-3′cyclic nucleotide-3 phosphodiesterase (CNP) and proteolipid protein (PLP), play significant roles in the myelin architecture. MBP is essential for the compaction of two cytoplasmic interfaces of myelin lamellae and formation of major dense line (Wolf et al. 2021). PLP, a transmembrane protein in CNS myelin, contributes to the tight apposition of the extracellular surfaces to each other forming a double intraperiod line and also mediates the closure of cytoplasmic channels (Snaidero and Simons 2014). CNP helps in maintaining the functional cytoplasmic-rich compartment in myelin and mediates the interactions with the actin cytoskeleton to keep the channels open (Snaidero et al. 2017). Other important CNS myelin proteins include myelin-associated glycoprotein (MAG) involved in axo-myelin interactions; myelin oligodendrocyte glycoprotein (MOG) located on the surface of the compacted myelin, involved in adhesion and interactions between adjacent sheaths within axon fascicles to provide structural integrity to myelin sheath; and myelin oligodendrocyte basic protein (MOBP) located in the major dense lines, which play a role in the compaction and stabilization of myelin (Montague et al. 2006; Pronker et al. 2016). Claudin-11 is essential for the formation of radial component of CNS myelin, which is a network of interlamellar tight junctions involved in mediating adhesion between myelin membranes and potentiates the insulative properties of myelin (Devaux and Gow 2008; Denninger et al. 2015).

About 70–75% of the dry weight of myelin is lipid, rich in saturated long-chain fatty acids that affect membrane thickness and the packing density of lipids in the myelin. Major myelin lipids are cholesterol, phospholipids, galactolipids and plasmalogens in the ratio of 2:2:1:1, respectively (Norton and Poduslo 1973; Schmitt et al. 2015). Cholesterol is an essential component of CNS myelin membranes and accounts for about 80% of the total brain cholesterol (Dietschy 2009). In the brain, cholesterol is not imported from the blood circulation; rather, it is synthesized by the cells of the brain and switches from neurons during embryogenesis to oligodendrocytes during postnatal life to astrocytes during adulthood (Morell and Jurevics 1996; Saher et al. 2015). The bulk of the cholesterol incorporated into the myelin is synthesized by oligodendrocytes. Thus, cholesterol is the only integral myelin component, and its availability in oligodendrocytes is a rate-limiting factor for brain maturation as shown in mutant mouse or zebrafish lacking enzymes essential for cholesterol synthesis (Saher 2005, 2015). Saher and associates (2015) also emphasized that cholesterol is involved in many aspects of myelin biogenesis and an interference with cholesterol homeostasis in the brain would affect the synthesis and maintenance of myelin. Myelination is also affected either directly or indirectly in disorders that interfere with synthesis or intracellular trafficking of cholesterol.

By using live in vivo imaging in zebrafish using electron microscopy, Snaidero and his associates, in 2014, explained the mechanism of myelin layer wrap around

the axons. They showed that the newly generated myelin layers settle by constant coiling of the innermost tongue of the myelin sheath over the axon along with the lateral spread of myelin along the axon. The dynamic changes in actin assembly facilitate the development of myelinic channels in uncompacted myelin that help in the transportation of metabolites from oligodendrocyte cytoplasm to the myelin and also to the neurons (Zuchero et al. 2015; Snaidero et al. 2017). Compromised metabolic support from oligodendrocytes to neurons causes the degeneration of certain neuronal subpopulations and neurodegenerative disorders. This suggests the importance of oligodendrocytes as metabolic supporters of neurons and neuronal homeostasis (Philips and Rothstein 2017).

Recent research suggests that myelination occurs in two phases: (a) genetically pre-defined intrinsic phase, occurring around birth to early childhood that proceeds in a precise spatiotemporal order, and (b) adaptive myelination driven as per the need of the neural network, which can be modified by experience leading to myelination variability in different individuals (Fields 2008; Chang et al. 2016; Mount and Monje 2017; Bechler et al. 2018). Adaptive myelination occurs more prominently in brain areas involved in complex behaviour, where the continuous myelination helps to fine-tune neuronal network function by synchronizing the firing pattern (De Hoz and Simons 2015; Filley and Fields 2016) and shape the myelination as per the requirement of the neuron and its network. Human MRI studies pointing to experience-based changes in WM and ultra-microscopic analysis of changes in myelin structure upon learning new tasks in animal models clearly indicate that myelination is influenced by experience (Oztürk et al. 2002; Bengtsson et al. 2005; Steele et al. 2013; McKenzie et al. 2014; Xiao et al. 2016). Thus, myelin deposition is a dynamic and plastic process showing adaptive changes in response to neuronal activity (Chang et al. 2016; Xiao et al. 2016; Gibson et al. 2014; Fields 2015). Neuregulin-ErbB signalling regulates the switch between intrinsic and adaptive myelination (Lundgaard et al. 2013). The oligodendrocytes are intrinsically capable of generating myelin, but the neuronal activity finally helps in adjusting the myelin thickness, suggesting that the role of electrical activity helps in myelin remodelling (Gibson et al. 2014). In addition, oligodendrocytes in different CNS regions have intrinsic differences (Crawford et al. 2016; Dimou and Simons 2017) with spinal cord oligodendrocytes forming longer sheath than cortical oligodendrocytes (Bechler et al. 2015).

Astrocytes also participate in the process of myelination by secreting soluble growth factors. Astrocyte-derived PDGF-α and leukaemia inhibitory factor (LIF)-like proteins are essential for the survival and differentiation of OPCs (Noble et al. 1988; McKinnon et al. 2005; Gard et al. 1995). In addition, astrocytes also transport lipids to oligodendrocytes for the generation of large myelin sheaths (Abrams 2017), and failure to this leads to persistent myelin deficits (Camargo et al. 2017). Astrocytes couple with oligodendrocytes through gap junctions formed by connexins and also with nodes of Ranvier and thus provide metabolic support to axons (Abrams 2017). Neonatal microglia promote myelination via insulin-like growth factor (Wlodarczyk et al. 2017).

Research information gathered during the last two decades suggests that in both humans and mice, the myelination is an ongoing process and continues throughout adult life either by generating new myelinating oligodendrocytes from their precursors (OPCs) or by remodelling of prevailing myelin to renew growth long after its initial formation for enhanced cell function (Young et al. 2013, 2014; Snaidero et al. 2014; Jeffries et al. 2016). Substantial changes in WM have been considered as a key regulatory factor for higher-order brain functions. This was well demonstrated in a mice model of social isolation stress imposed during critical period of postnatal development and myelination or beyond adulthood, which resulted in defects in myelination and associated behaviour (Makinoden et al. 2012; Liu et al. 2012), while motor learning in animal models was seen to promote the differentiation of adult OPCs and myelination (McKenzie et al. 2014). The proliferation of OPCs/NG2^{+} glia is also intensified in conditions of demyelination, traumatic injury to CNS and chronic neurodegenerative diseases (Magnus et al. 2008; Kang et al. 2010). The WM volume also decreases gradually with normal ageing (Sowell et al. 2003), and the frequent observations are changes in myelinated nerve fibre morphology and degenerative changes in myelin sheaths (Peters 2002). The ageing WM is extremely vulnerable to degeneration and loss of myelin. In ageing humans, the loss of myelin integrity correlates well with the decline of cognitive functions (Bastin et al. 2010). Although the WM degeneration is not uniform throughout the CNS with the association tracts more susceptible to myelin loss than the projection tracts, the myelin deficits in the corpus callosum are commonly seen in all men and women at old age and might be a crucial factor for age-associated impairment in cognition and working memory (Sullivan et al. 2001; Kohama et al. 2012; Hedden et al. 2016). Other factors like nutritional deficiencies, early life infections and stress have also been found to cause myelin deficits. In an intra-generational protein deprivation rat model, we have also reported drastic changes in oligodendrogenesis in terms of reduction in oligodendrocyte progenitor pool, reduced expression of myelin genes leading to hypo-myelination, disorganized myelin fibre alignments, reduced corpus callosum calibre and associated behavioural deficits persisting through pre-adolescence to late adulthood (Patro et al. 2019). Early life exposure to LPS-induced bacterial infection also results in demyelinating changes during adulthood and senility by altering the expression of myelin proteins resulting in motor coordination deficits (Singh et al. 2017).

Defects in Myelination and Neuropathologies

Developmental myelination and remyelination in adult CNS are high energy-demanding tasks as the synthesis of myelin sheath requires vast quantities of lipids and proteins (Baron and Hoekstra 2010). To meet this demand, oligodendrocytes consume a large amount of metabolites, like glucose and lactate, which are supplied from the diet or from the stored protein, fat and glycogen source, which needs to be continuously refuelled. This high energy demand imposed on oligodendrocytes makes them susceptible to oxidative stress, cytotoxic by-products and excitotoxic factors leading to their altered functions (Juurlink et al. 1998; Matute et al. 1997, 2007). Oligodendrocyte pathology is clearly seen in many disorders like

Alzheimer's disease, multiple sclerosis (MS), schizophrenia, traumatic injuries and ischaemia, some leukodystrophies or autoimmune attacks and demyelinating disorders (Fancy et al. 2011; Traka et al. 2016; Torii et al. 2014; Duncan and Radcliff 2016; McAleese et al. 2017). In addition, the ageing is also associated with WM atrophy, gradual impairment in motor learning and reduced remyelination ability (Sim et al. 2002; Ruckh et al. 2012).

Myelin has recently been reported to provide metabolic support to neurons crucial for axonal integrity and neuronal activity, indicating its persistent role in neural circuit formation and functions throughout life (Saab et al. 2016). This emphasizes that disruption of myelin and myelin-forming oligodendrocytes in CNS may have significant neurological manifestations and may lead to neurodevelopmental, neurodegenerative and neuropsychiatric pathologies, viz. leukodystrophies, schizophrenia, multiple sclerosis, amyotrophic lateral sclerosis and others (Compston and Coles 2008; Franklin et al. 2012; Pouwels et al. 2014; Jin et al. 2015; Miyata et al. 2015). Multiple factors have been reported to variously affect the process of myelination, notably its proportion and timing. Intra-generational protein malnutrition negatively affects overall development of the brain, oligodendrocyte development and maturation, impaired myelination and motor deficits at adolescence and later (Patro et al. 2019). Other nutritional deficiencies, viz. acquired or genetic B_{12} or folate deficiencies, also lead to delayed myelination or even white matter disturbances, cortical insults and peripheral neuropathy (Prado and Dewey 2014; Kobayashi et al. 2016). Thyroid hormone is required for the terminal differentiation of OPCs into myelinating cells, and thus hypothyroidism also leads to delayed myelination (Barres et al. 1994; Lee and Petratos 2016). In addition, early life exposure to viral (our data under publication) and bacterial infections also leads to demyelinating lesions in the adult brain leading to poor motor coordination (Singh et al. 2017).

Myelin diseases are generally grouped into three types, demyelinating, dysmyelinating and hypomyelinating disorders, caused by loss of myelin, abnormal myelin production or compromised myelin production, respectively. In demyelinating disorders, the myelin loss may occur either due to direct damage to myelin sheath or indirectly by disruption or death of OLs consequent upon inflammation or toxic insults and also by axonal injury through Wallerian degeneration. The dysmyelinated diseases primarily occur due to genetic defects involving intrinsic abnormalities in the production and maintenance of myelin and appear early in life during childhood or adolescence. In the hypomyelinating conditions, the axons are either unmyelinated or myelinated with thin myelin sheaths, mainly due to the genetic defects or epigenetic adversities. The myelin pathologies have also been categorized into inherited and acquired based on their origin whether genetic, inflammatory or toxic (Duncan and Radcliff 2016). Antibody-mediated diseases, primary axonal pathologies and structural protein defects are preferentially associated with myelin damage, while viral infection and genetic and metabolic deficiencies negatively influence OL survival.

Primary Demyelinating Diseases

Multiple Sclerosis

Multiple sclerosis (MS) is the most common and well-researched acquired demyelinating human disease affecting CNS myelin. It affects individuals of all age groups but most prevalent in young adults (Reich et al. 2018) and forms the foremost cause of nontraumatic neurological disability in the young and middle-aged population. The occurrence is 2.3 times more prevalent in females, and the susceptibility is strongly related to the human leukocyte antigen (HLA) locus, and homozygosity at the HLA-DRB1*15 gene locus increases the incidence to develop the disease (Stadelmann et al. 2019). Moreover, the polymorphism in genes regulating both the innate and adaptive immunity, regulatory and cytotoxic T-cell and microglia functions present higher risk of disease (Sawcer et al. 2011).

MS is mediated by autoreactive immune cells, myelin-specific $CD8^+$ T cells, targeting the myelin and the OLs, initiating myelin damage and axonal injury and leading to sensory-motor and/or visual impairments (Noseworthy et al. 2000; Trapp and Nave 2008; Filippi et al. 2018). Clinically, the disease may be classified into relapsing-remitting MS, primary progressive MS and secondary progressive MS. The hallmarks of MS are demyelinating lesions in the CNS, characterized by immune cell infiltration across the blood-brain barrier (BBB), triggering an inflammatory response, myelin damage, activation of astrocytes and microglia and axonal injury (Matveeva et al. 2018). With disease progression, more striking grey and white matter lesions are seen with the persistent gliosis throughout the course of the disease (Dendrou et al. 2015). Moreover, in the MS patients, the components of the myelin sheath, like MBP, MOG and PLP, have been identified as autoantigens mainly by the $CD4^+$ T cells (Androutsou et al. 2018).

Following focal demyelination, the consequences of the autoimmune attack are progressive, leaving the axons exposed to toxic environment, inefficient and vulnerable to degeneration, ultimately leading to axonal loss and neuronal death, a pathological hallmark of MS (De Stefano et al. 1998; Bradl and Lassmann 2010; Duncan and Radcliff 2016). Metabolic dysfunction is commonly associated with the pathogenesis of MS along with impaired mitochondrial functioning, oxidative stress resulting in axonal energy failure and subsequent neurodegeneration (Adiele and Adiele 2017). Impaired energy metabolism also leads to oligodendrogliopathy by either lack of blood supply to lesions or the production of toxic metabolites (Lassmann 2003).

Remyelination is triggered during the early phase of the disease by the generation of new mature OLs, which help in restoring the myelin to sheath the denervated axons and recover the saltatory conduction, axonal integrity and functional deficits (Kierstead and Blakemore 1999; Franklin and French-Constant 2008). Recently, Duncan and associates (2018) have indicated that mature OLs can also help in remyelination. However, in later progressive phase of MS, remyelination is not sufficient to recondition the severe demyelination, leading to remyelination failure and exacerbating to chronic relapsing progressive MS (Franklin 2002; Goldenberg

2012; Gruchot 2019). There are two potential sources of myelinating OPCs that participate in the remyelination of MS axons: the parenchymal OPCs and the endogenous NSCs. Parenchymal OPCs are more sensitive to endogenous growth factors produced by resident CNS cells than the mature OLs. The proliferation, differentiation and maturation of the OPCs to mature OLs are strongly influenced by the extracellular milieu and crucial to regain homeostasis and replace the lost OLs (Duncan et al. 2021). But the absence of the appropriate signalling factors in the MS environment inhibits the differentiation of OPCs to mature OLs and prevents successful remyelination (Franklin 2002; Gruchot 2019). Moreover, poor clearance and accumulation of the myelin debris at the MS lesion sites may also impair the differentiation of oligodendroglial lineage cells (Kotter et al. 2006; Baer et al. 2009).

Multiple molecular pathways have been identified that block the differentiation of OPCs into mature OLs. These pathways are potentially targeted to explore therapeutic interventions to promote repair. Some of these pathways include the dysregulation of Wnt pathway (Fancy et al. 2009), signalling through glycosaminoglycan hyaluronan that accumulates at the site of MS lesions (Back et al. 2005; Sloane et al. 2010) and axonally derived neuregulin (Vartanian et al. 1999) that prevents the differentiation of OL precursors and contributes to the remyelination failure. Chronic metabolic stress in MS lesions also contributes to remyelination failure because of OPC dysfunction and dying back of the OL terminal processes leading to the destabilization of myelin/axon interactions (Rone et al. 2016). Together, these findings suggest that the impaired differentiation of OPCs is the major cause of remyelination failure in MS, not the deficiency of OPCs (Kuhlmann et al. 2008).

There are many diseases that are associated with MS and are listed under demyelinating disorders: (a) neuromyelitis optica (Devic's disease), a variant of MS or a separate demyelinating disease which primarily attacks the optic nerve and spinal cord; (b) Balo's disease (concentric sclerosis), a rare demyelinating disorder in which the CNS myelin is damaged; (c) Schilder's disease (diffuse sclerosis), an acute rapidly progressive, degenerative, demyelinating disease of the CNS, seen in childhood; and (d) Marburg's disease, a variant of MS, characterized as acute, fatal, fulminant or malignant MS showing acute demyelination.

Several murine models mimicking MS have been developed, viz. (1) inflammation-dependent, experimental autoimmune encephalitis (EAE) and viral encephalomyelitis and (2) inflammation-independent, chemical and toxin-induced demyelination using EtBr, lysolecithin, cuprizone, etc. These models have been useful in replicating various clinical, immunological and microscopic aspects of the human MS and helped to understand the role of immune components in CNS myelination and repair as well as the complex nature of the CNS environment to enable to develop novel therapeutic modalities (Miller et al. 2001; Bergmann et al. 2006; Mecha et al. 2013; Borjini et al. 2016).

Secondary Demyelinating Diseases

Acute Disseminated Encephalomyelitis (ADEM)

ADEM is an acute inflammatory demyelinating disease of the CNS, caused mainly due to the exposure of antigens through upper respiratory and gastrointestinal infections or after immunizations (Scolding 2014). Clinicopathological features of ADEM show multiple demyelinating lesions confined to the perivenular tissue in specific brain regions, with minimal signs of progressive neurodegeneration that are typical of MS, suggesting different pathogenic mechanisms of the two diseases. The perivascular inflammatory infiltrate consists of T cells, foamy macrophages, granulocytes and eosinophils (Hart and Earle 1975; Young et al. 2010).

Although the mechanism of ADEM pathogenesis is not clear, antibody reactivity against myelin proteins has been found to be critical. Antigens such as MBP, PLP and MOG are the targets of the reactive T cells, as was revealed from a study reporting the presence of IgG antibodies reacting to various myelin proteins in the CSF of patients (Cole et al. 2019). IgG antibodies against MOG are frequently found in the serum of patients with inflammatory demyelination and paediatric ADEM (Mader et al. 2011; Hoftberger and Lassmann 2017), and thus MOG detection serves as a reliable diagnostic marker of these diseases. MOG antibodies initiate demyelination and cell death by activating the complement cascade and contribute to the disease pathogenesis. Perivascular demyelinating lesions are usually surrounded by macrophages containing residual myelin proteins and infiltrates of T and B cells, granulocytes, plasma cells and activated astrocytes and microglia (Scolding 2014; Popescu and Lucchinetti 2011). Axons are mainly spared, but finally show features of injury during acute demyelination in ADEM, and the exudate from the wall of the blood vessels leads to necrosis of the neighbouring tissue showing the conjoining features of ADEM and acute haemorrhagic leukoencephalopathies (Hoftberger and Lassmann 2017). Most patients respond to treatment and recover, but others survive with mild to moderate neurological deficits (Cole et al. 2019).

Neuromyelitis Optica (NMO)/Neuromyelitis Optica Spectrum Disorder (NMOSD)

NMO also known NMOSD or Devic's syndrome is a severe inflammatory demyelinating disease of unknown aetiology that predominantly affects the optic nerves and spinal cord, causing blindness and motor paralysis (Lennon et al. 2004; Weinshenker and Wingerchuk 2017). The disease is basically allocated to AQP4 serum antibodies (NMO-IgG) that target primarily the astrocytes, progressing to oligodendrocyte and myelin damage as well as substantial axonal loss (Misu et al. 2007; Parratt and Prineas 2010; Wingerchuk et al. 2015). The disease pathogenesis involves the potential of anti-AQP4 antibodies causing the local activation of the complement system leading to astrocyte death and early loss of oligodendrocytes and OPCs, subsequently leading to loss of myelin and formation of NMO typical lesions, distinct from MS (Parratt and Prineas 2010; Wrzos et al. 2014; Tradtrantip et al. 2017). The near-complete absence of astrocytes and oligodendrocytes in the

NMO lesions fails to support the oligodendrocyte regeneration and myelin repair (Parratt and Prineas 2010). Axons are preserved in early NMO lesions, but in the chronic disease stage, axon loss and spinal cord atrophy are usually intense (Wrzos et al. 2014; Herwerth et al. 2016).

Leukodystrophies

Demyelination Due to Mutations and Defects in Oligodendrocyte- and Myelin-Related Genes

Genetic mutations in OL-specific genes, viz. the genes encoding structural myelin proteins, enzymes involved in various metabolic pathways and proteins involved in myelin development and maintenance, are associated with hypomyelination, dys−/demyelination or myelin swelling (van der Knapp and Bugiani 2017).

The *Pelizaeus-Merzbacher disease (PMD)* and *X-linked severe spastic paraplegia (SPG2)* are inherited myelin disorders caused by mutation in *PLP1 gene* that encodes for the structural myelin membrane protein, PLP, and its alternatively spliced form DM20. About 60–70% of the PMD patients show duplication of *PLP1* gene, while the rest of the PMD cases reveal missense or point mutations, insertions and deletions (Torii et al. 2014). As PLP is essentially required for the apposition of the myelin sheaths, any defects in its expression may be deleterious for the CNS functioning. PLP duplication generally causes classical PMD with patients showing hypomyelination in the cerebral and cerebellar WM, while the grey matter is comparatively preserved (Harding et al. 1995). Mature OLs are completely absent, because of massive apoptosis due to the altered myelin membrane composition, abnormal level and accumulation of PLP and associated lipids and toxic gain in function in OLs (Simons et al. 2002; Karim et al. 2007; Sima et al. 2009; Torii et al. 2014). PLP null mutations also result in hypomyelination, but the myelin loss is much less than the PLP duplication in PMD patients. Although the WM appears to be well myelinated, axonal swelling and Wallerian degeneration are frequently present (Garbern et al. 2002; Sima et al. 2009). This suggests that axonal survival is crucially dependent on proper myelin function (Nave and Werner 2014). Moreover, several point mutations in *PLP1* gene have also been reported to cause demyelination and oligodendrocyte loss ranging from mild to severe connatal forms (Calloux et al. 2000; Hübner et al. 2005).

The *Pelizaeus-Merzbacher-like disease (PMLD)* is a recessive inherited demyelinating disease caused by mutations in *GJC2* gene encoding the OL-specific connexin, Cx47, involved in gap junctional communication for myelin development and maintenance (Menichella et al. 2003; Abrams and Orthmann-Murphy 2013). Demyelination in PMLD patients occurs due to the loss of function of gap junctional communication in mature oligodendrocytes, oligodendrocyte apoptosis and defective myelin development and maintenance.

Mutations in genes related to lipid metabolism are also associated with many leukodystrophies. Metachromatic leukodystrophy (MLD) is an autosomal recessive leukodystrophy caused by mutation in *ARSA* gene encoding for the lysosomal protein arylsulphatase A (ASA) or mutation in PSAP gene encoding prosaporin,

an activator of ASA (Cesani et al. 2016). ASA plays a crucial role in the metabolism of sulphatides, a major myelin lipid, and the defects in its metabolism lead to its impaired degradation and intra-lysosomal accumulation in the nervous system and other visceral organs (Eckhardt 2008). Lipid accumulation directly causes the demyelination leading to the death of OLs accompanied by myelin destruction and severe axonal damage (Kohlschütter 2013). However, the pathophysiological mechanisms linking the metabolic alterations and myelin abnormalities with axonal dysfunction are still ambiguous.

Mutations in Microglial and Astrocytic Genes and Leukodystrophies

Microglia, the resident immune cells of the CNS, are now well documented to play a role in myelin homeostasis and development as is clear from two hereditary diseases, diffuse leukoencephalopathy with spheroids (HDLS) and the Nasu-Hakola disease (NHD), caused by mutations in microglial genes. The affected genes [colony-stimulating factor 1 receptor (CSF1R) in HDLS and TREM2 and DAP12 in NHD] involved in both the diseases are key regulators of microglial activation and neuroinflammatory pathways (Rademakers et al. 2012; Paloneva et al. 2000, 2001). Clinically both the diseases present similar features with demyelination and axonal loss in defined brain areas, axonal swellings and spheroids, myelin vacuolization in demyelinating areas, widespread astrogliosis and tau-positive neurites in the cortex (Baba et al. 2006; Paloneva et al. 2001; Robinson et al. 2015). The mechanism linking the microglial dysfunction and demyelination is not clearly resolved. However, there are reports that suggest that demyelination might arise as a consequence of primary neuroaxonal damage and microglial dysfunction, impaired OPC homeostasis and reduced microglial number affecting myelin clearance and persistent demyelination (Stadelamnn et al. 2019).

Research in the last decade has revealed a highly specialized role of astrocytes in the physiology and pathology of the nervous system ranging from protective to destructive role (Pekny et al. 2016). Although there is only scanty information about the role of astrocytes in OL health and myelin development, maintenance and pathology, the demyelinating disorders caused by the astrocyte-specific genes clearly indicate the intercellular interactions between these two cell types. Mutation in GFAP gene, encoding an intermediate filament protein expressed in astrocytes, causes an autosomal dominant disorder, Alexander disease. GFAP overexpression might be involved in the pathogenesis of Alexander disease through gain-of-toxic function of GFAP and astrocyte dysfunction (Messing et al. 1998). The mechanism linking the astrocyte dysfunction with demyelination might include (a) the increased expression of CXCL10 in astrocytes that may directly affect OLs or exert immune response triggering demyelination (Olabarria and Goldman 2017), (b) loss of gap junctional communication and altered buffering capacity of astrocytes that may lead to intramyelinic oedema (Sosunov et al. 2013) and (c) extracellular deposition of hyaluronan, an astrocytic protein that deposits in extracellular space and might inhibit OPC differentiation and maturation (Bugiani et al. 2013). More recent research proposed that GFAP mutation defects may directly interfere in the cellular

development and OPC differentiation from neural progenitor cells expressing GFAP (Gomez-Pinedo et al. 2017).

Vanishing white matter disease (VWM), which is one of the most prevalent leukodystrophies with a central role of astrocytes in its pathogenesis, mainly affects the CNS. The disease is inherited in an autosomal recessive manner and is characterized by childhood onset chronic neurological deterioration, signified by cerebellar ataxia. It is caused by mutation in any of the genes encoding the five subunits of the eukaryotic translation initiation factor 2B (eIF2B; Bugiani et al. 2011, 2018). Defective maturation and dysfunction of astrocytes due to abnormal composition of its cytoskeletal protein GFAP and an upregulation in the heat shock protein αB-crystallin along with the co-existence of increased density of pre-myelinating OL progenitors might be involved in the loss of WM in VWM (Bugiani et al. 2011). In their later publication, Bugiani and his group (2018) stressed that the astrocytes play a central role in the pathogenesis of VWM with the secondary effects on oligodendrocytes and axons; thus, VWM may be grouped with astrocytopathies in which the loss of essential function and gain of detrimental function by astrocytes could be driving the WM degeneration and VWM pathogenesis (van der Knapp and Bugiani 2017).

Viral Encephalopathies

The most common human demyelinating diseases with viral aetiology include progressive multifocal leukoencephalopathy (PML), acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) and subacute sclerosing panencephalitis (SSPE). The common mechanism of demyelination in most viral CNS infections involves OL damage/loss and the breakdown of the cellular machinery required for myelin synthesis.

PML is the most studied fatal demyelinating disease caused by JC virus (JCV) infection, a double-stranded DNA polyomavirus, commonly seen to affect the CNS of individuals on immune-modulatory therapies (Major 2010). JCV induces demyelination by infection, followed by lysis of the OLs, which subsequently infects the surrounding OLs and results in focal demyelination. The infected OLs contain inclusion bodies with viral particles in the nuclei and show nuclear swelling and loss of chromatin leading to their demise (Richardson 1961). The neurons and astrocytes are also infected to some extent (Wollebo et al. 2015). The infected astrocytes appear hypertrophied with irregular and lobulated nuclei and give bizarre appearance, and their presence in areas without apparent demyelinating lesions might be involved in viral propagation (Seth et al. 2004). Demyelination finally results in axonal dysfunction leaving the axons susceptible to toxic products released by the surrounding glial cells causing the retrograde loss of neuronal cell body. Microglia and macrophages are not infected by JCV but are seen in the centre of the demyelinating lesions (Ferenczy et al. 2012).

The patients with AIDS show tendency to develop multiple CNS infections with high incidence to develop PML. The pathogenesis of HIV-associated encephalomyelitis may involve an immune-mediated "bystander effect" with myelin destruction via cytokines released by activated monocytes and lymphocytes (Corral et al. 2004).

In HIV-PML patients, massive necrotic demyelinating lesions are seen with the infiltration of HIV-infected macrophages and microglia (Wiley et al. 1988). Persistent viral infection caused by defective measles virus causes another progressive neurological disorder known as SSPE, a progressive fatal demyelinating disease. The virus destroys the host cells, including oligodendrocytes, and initiate inflammatory responses resulting in demyelination.

Vascular (Hypoxia/Ischaemia)

WM abnormalities in the elderly individuals usually result from ischaemia, secondary to the damage of the cerebral arteries. The degree of WM abnormality directly correlates with the impaired motor and cognitive abilities. Other vascular WM abnormalities include postanoxic encephalopathy developed after a severe anoxic episode, reversible posterior leukoencephalopathy and Binswanger's disease.

Metabolic/Nutritional

Alcoholism, malnutrition, disability and other debilitating conditions also lead to the demyelination, commonly called as central pontine myelinolysis involving severe damage to the myelin sheath of nerve cells in the pons. This condition is also known to be associated with liver or kidney failure, diabetes mellitus, immunosuppressive therapy and long-term usage of some other drugs. Demyelination is usually seen without inflammatory response sparing the blood vessels, most neurons and axons. Alcoholism and nutritional deficiencies are also known to cause sustained demyelination of the corpus callosum. Thiamine deficiency leads to severe memory impairment and anterograde amnesia, a hallmark of the Wernicke encephalopathy.

Other Concerns for Demyelination

A variety of chemical substances are also known to damage myelin sheath or OLs or both. Most of them are known as myelinotoxic or glia toxic chemicals and include hexachlorophene (HCP), triethyltin (TET), lysolecithin, ethidium bromide, zymosan and cuprizone. Most of them are used experimentally to create animal models, but some of them are known to affect human beings through exposure (Duncan and Radcliff 2016). There are other toxins and nutritional deficiencies that cause myelin vacuolation disorders. Most of the toxin are used experimentally and include cycloleucine, sodium cyanate, actinomycin D, isoniazid, 6-amino-recotinamide, etc. In addition, myelin vacuolation is also noted in cases of vitamin B12 deficiency, genetic deletion of Cx30 or Cx40, knockout of the enzyme UDP-galactose:ceramide galactosyltransferase and duplication of laminin 1 gene in mouse.

Traumatic injury of the brain (TBI) or spinal cord can also lead to death of oligodendrocytes and demyelination (Plemel et al. 2014; Mierzwa et al. 2015). In both the cases, damage to the myelin may be caused by ischaemic effect on OLs primarily due to excitotoxicity and glutamate elevation (Tsutsui and Stys 2013). Radiation therapy commonly used to treat brain tumours can also cause demyelination by killing the OPCs (Panagiotakos et al. 2007). In addition, there are a variety of other demyelinating diseases and WM disorders in which myelin is damaged and are being increasingly identified because of the modern imaging techniques and the

next-generation sequencing to identify the mutant gene; these are beyond the scope of this chapter.

Remyelination Strategies

As detailed vide supra, the disruption of OLs or the myelin sheath following injury and disease bears direct consequences on the function of neurons. In addition to demyelination, long-term effects of OL death include axonal atrophy and neuronal loss and form the major cause of many neurological disorders, including MS, inherited leukodystrophies of the CNS and the neuropathies of peripheral nervous system (PNS) (Nave 2010). Demyelination is a common factor in most of these diseases, thus becoming a therapeutic target with enormous potential. However, the endogenous myelin repair/remyelination will occur in a long time (Duncan et al. 2020). Moreover, in the remyelinated tissue, the myelin sheaths are shorter and thinner with lower conduction amplitude than the developmental myelin sheath (Gallo and Deneen 2014; Almeida 2018). Although the endogenous remyelination does occur in both CNS and PNS, the process is much less impressive in CNS (Franklin and French-Constant 2008). Moreover, remyelination being a time-consuming process, acute demyelination can finally lead to cell death before the remyelination happens. OPCs are the largest source of endogenous progenitors in CNS. During remyelination, the OPCs become multipotent and proliferative, migrate to the site of lesion, exit the cell cycle and differentiate into myelin producing OLs (Silveira et al. 2021). Although the mature OLs in remyelination have also been reported (Duncan et al. 2018; Macchi et al. 2020), their contribution is still a matter of speculation and needs further clarification. Thus, OPCs provide the majority of remyelination. Moreover, there are many barriers of endogenous remyelination that limit the regeneration in the CNS. Thus, treatments and therapies targeting OLs and remyelination may have widespread potential for application. Most treatment strategies used are targeted to prevent progressive demyelination that contributes to chronic disability, but a few can promote remyelination. Various translational approaches have been devised using several animal species and models for TBI/SCI and MS. These include (a) extrinsic and intrinsic factors that act as either the inhibitors or stimulators of OPC differentiation (Gruchot et al. 2019); (b) use of monoclonal antibodies targeted to enhance axonal regeneration and suppress neuroinflammation and against the endogenous CNS myelin inhibitory molecules; (c) a gene therapy RNA interference approach; and (d) cell replacement strategies. However, the human trials are very limited with poor success rate so far.

Acknowledgements The Department of Biotechnology's support through the DBT-National Initiative on Glial Cell Research in Health and Disease is gratefully acknowledged. We are thankful to the Department of Biotechnology, the Department of Science and Technology and the Indian Council of Medical Research, Govt. of India, for their financial support through various grants. We are thankful to Dr. Urmishree Bedamatta for critically evaluating the manuscript for its English.

References

Abrams CK (2017) Diseases of connexins expressed in myelinating glia. Neurosci Lett 695:91–99
Abrams CK, Orthmann-Murphy J (2013) Connexin mutations in Pelizaeus-Merzbacher-like disease, oculodentodigital dysplasia and related diseases. In: Dere E (ed) Gap junctions in the brain. Academic, San Diego, pp 165–187
Adiele RC, Adiele CA (2017) Metabolic defects in multiple sclerosis. Mitochondrion 44:7–14. https://doi.org/10.1016/j.mito.2017.12.005
Aggarwal S, Yurlova L, Snaidero N, Reetz C, Frey S, Zimmermann J, Pähler G, Janshoff A, Friedrichs J, Müller DJ, Goebel C (2011) A size barrier limits protein diffusion at the cell surface to generate lipid-rich myelin-membrane sheets. Dev Cell 21:445–456
Almeida R, Lyons D (2016) Oligodendrocyte development in the absence of their target axons in vivo. PLoS One 11:e0164432
Almeida RG (2018) The rules of attraction in central nervous system myelination. Front Cell Neurosci 12:367. https://doi.org/10.3389/fncel.2018.00367
Androutsou ME, Tapeinou A, Vlamis-Gardikas A, Tselios T (2018) Myelin oligodendrocyte glycoprotein and multiple sclerosis. Med Chem 14:120–128. https://doi.org/10.2174/1573406413666170906123204
Asato MR, Terwilliger R, Woo J, Luna B (2010) WM development in adolescence: a DTI study. Cereb Cortex 20:2122–2131. https://doi.org/10.1093/cercor/bhp282
Baba Y, Ghetti B, Baker MC, Uitti RJ, Hutton ML, Yamaguchi K, Bird T, Lin W, De Lucia MW, Dickson DW, Wszolek ZK (2006) Hereditary diffuse leukoencephalopathy with spheroids: clinical, pathologic and genetic studies of a new kindred. Acta Neuropathol 111:300–311. https://doi.org/10.1007/s00401-006-046-z
Back SA, Tuohy TM, Chen H, Wallingford N, Craig A, Struve J, Luo NL, Banine F, Liu Y, Chang A, Trapp BD, Bebo BF Jr, Rao MS, Sherman LS (2005) Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. Nat Med 11:966–972
Baer AS, Syed YA, Kang SU, Mitteregger D, Vig R, Ffrench-Constant C, Franklin RJ, Altmann F, Lubec G, Kotter MR (2009) Myelin-mediated inhibition of oligodendrocyte precursor differentiation can be overcome by pharmacological modulation of Fyn-RhoA and protein kinase C signalling. Brain 132:465–481
Baron W, Hoekstra D (2010) On the biogenesis of myelin membranes: sorting, trafficking and cell polarity. FEBS Lett 584:1760–1770
Barres BA, Lazar MA, Raff MC (1994) A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. Development 120:1097–1108
Barres BA, Raff MC (1993) Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. Nature 361:258–260
Bastin ME, Munoz Maniega S, Ferguson KJ, Brown LJ, Wardlaw JM, MacLullich AM, Clayden JD (2010) Quantifying the effects of normal ageing on white matter structure using unsupervised tract shape modelling. Neuroimage 51:1–10
Bechler ME, Byrne L (2015) CNS myelin sheath lengths are an intrinsic property of oligodendrocytes. Curr Biol 25:2411–2416
Bechler ME, Swire M, Ffrench-Constant C (2018) Intrinsic and adaptive myelination-a sequential mechanism for smart wiring in the brain. Dev Neurobiol 78:68–79. https://doi.org/10.1002/dneu.22518
Bengtsson SL, Nagy Z, Skare S, Forsman L, Forssberg H, Ullén F (2005) Extensive piano practicing has regionally specific effects on white matter development. Nat Neurosci 8:1148–1150. https://doi.org/10.1038/nn1516
Bergmann CC, Lane TE, Stohlman SA (2006) Coronavirus infection of the central nervous system: host–virus stand-off. Nat Rev Microbiol 4:121–132. https://doi.org/10.1038/nrmicro1343
Birchmeier C, Nave KA (2008) Neuregulin-1, a key axonal signal that drives Schwann cell growth and differentiation. Glia 56:1491–1497

Borjini N, Fernández M, Giardino L, Calzà L (2016) Cytokine and chemokine alterations in tissue, CSF, and plasma in early presymptomatic phase of experimental allergic encephalomyelitis (EAE), in a rat model of multiple sclerosis. J Neuroinflammation 13:291. https://doi.org/10.1186/s12974-016-0757-6

Bradl M, Lassmann H (2010) Oligodendrocytes: biology and pathology. Acta Neuropathol 119:37–53

Braun PE, Sandillon F, Edwards A, Matthieu JM, Privat A (1988) Immunocytochemical localization by electron microscopy of 2′,3’-cyclic nucleotide 3’-phosphodiesterase in developing oligodendrocytes of Normal and mutant brain. J Neurosci 8:3057–3066

Brinkmann BG, Agarwal A, Sereda MW, Garratt AN, Müller T, Wende H, Stassart RM, Nawaz S, Humml C, Velanac V, Radyushkin K (2008) Neuregulin-1/ErbB signalling serves distinct functions in myelination of the peripheral and central nervous system. Neuron 59:581–595

Brody BA, Kinney HC, Kloman AS, Gilles FH (1987) Sequence of central nervous system myelination in human infancy- an autopsy study of myelination. J Neuropathol Exp Neurol 46:283–301

Bugiani M, Boor I, van Kollenburg B, Postma N, Polder E, van Berkel C, van Kesteren RE, Windrem MS, Hol EM, Scheper GC, Goldman SA (2011) Defective glial maturation in vanishing white matter disease. J Neuropathol Exp Neurol 70:69–82

Bugiani M, Postma N, Polder E, Dieleman N, Scheffer PG, Sim FJ, van der Knaap MS, Boor I (2013) Hyaluronan accumulation and arrested oligodendrocyte progenitor maturation in vanishing white matter disease. Brain 136:209–222

Bugiani M, Vuong C, Breur M, van der Knaap Marjo S (2018) Vanishing white matter: a leukodystrophy due to astrocytic dysfunction. Brain Pathol 2018:408–421

Buyanova IS, Arsalidou M (2021) Cerebral White matter myelination and relations to age, gender, and cognition: a selective review. Front Hum Neurosci 15:662031. https://doi.org/10.3389/fnhum.2021.662031

Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, Thompson WJ (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci 28:264–278

Cai J, Qi Y, Hu X, Tan M, Liu Z, Zhang J, Li Q, Sander M, Qiu M (2005) Generation of oligodendrocyte precursor cells from mouse dorsal spinal cord independent of Nkx6 regulation and Shh signaling. Neuron 45:41–53

Cailloux F, Gauthier-Barichard F, Mimault C, Isabelle V, Courtois V, Giraud G, Dastugue B, Boespflug-Tanguy O (2000) Genotype-phenotype correlation in inherited brain myelination defects due to proteolipid protein gene mutations. Clinical European network on brain Dysmyelinating disease. Eur J Hum Genet 8:837–845

Calver AR, Hall AC, Yu WP, Walsh FS, Heath JK, Betsholtz C, Richardson WD (1998) Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron 20:869–882

Camargo N, Goudriaan A, van Deijk AL, Otte WM, Brouwers JF, Lodder H, Gutmann DH, Nave KA, Dijkhuizen RM, Mansvelder HD, Chrast R (2017) Oligodendroglial myelination requires astrocyte-derived lipids. PLoS Biol 15:e1002605

Castelfranco AM, Hartline DK (2015) The evolution of vertebrate and invertebrate myelin: a theoretical computational study. J Comput Neurosci 38:521–538

Cesani M, Lorioli L, Grossi S, Amico G, Fumagalli F, Spiga I, Filocamo M, Biffi A (2016) Mutation update of ARSA and PSAP genes causing metachromatic Leukodystrophy. Hum Mutat 37:16–27. https://doi.org/10.1002/humu.22919

Chang KJ, Redmond SA, Chan JR (2016) Remodeling myelination: implications for mechanisms of neural plasticity. Nat Neurosci 19:190–197. https://doi.org/10.1038/nn.4200

Cole J, Evans E, Mwangi M, Mar S (2019) Acute disseminated encephalomyelitis in children: an updated review based on current diagnostic criteria. Pediatr Neurol 100:26–34. https://doi.org/10.1016/j.pediatrneurol.2019.06.017

Compston A, Coles A (2008) Multiple sclerosis. Lancet 372:1502–1517

Corral I, Quereda C, García-Villanueva M, Casado JL, Pérez-Elías MJ, Navas E, Ariza A, Moreno S (2004) Focal monophasic demyelinating leukoencephalopathy in advanced HIV infection. Eur Neurol 52:36–41

Craig A, Luo NL, Beardsley DJ, Wingate-Pearse N, Walker DW, Hohimer AR, Back SA (2003) Quantitative analysis of perinatal rodent oligodendrocyte lineage progression and its correlation with human. Exp Neurol 181:231–240

Crawford AH, Tripathi RB, Richardson WD, Franklin RJ (2016) Developmental origin of oligodendrocyte lineage cells determines response to demyelination and susceptibility to age-associated functional decline. Cell Rep 15:761–773

Davis AA, Temple S (1994) A self-renewing multipotential stem cell in embryonic rat cerebral cortex. Nature 372:263–266

Dawson MR, Polito A, Levine JM, Reynolds R (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 24: 476–488

De Hoz L, Simons M (2015) The emerging functions of oligodendrocytes in regulating neuronal network behaviour. Bioessays 37:60–69. https://doi.org/10.1002/bies.201400127

De Stefano N, Matthews PM, Fu L, Narayanan S, Stanley J, Francis GS, Antel JP, Arnold DL (1998) Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. Brain 121(8): 1469–1477

Dean JM, Moravec MD, Grafe M, Abend N, Ren J, Gong X, Volpe JJ, Jensen FE, Hohimer AR, Back SA (2011) Strain-specific differences in perinatal rodent oligodendrocyte lineage progression and its correlation with human. Dev Neurosci 33:251. https://doi.org/10.1159/000327242

Dendrou CA, Fugger L, Friese MA (2015) Immunopathology of multiple sclerosis. Nat Rev Immunol 15:545–558. https://doi.org/10.1038/nri3871

Denninger AR, Breglio A, Maheras KJ, LeDuc G, Cristiglio V, Deme B, Gow A, Krischner DA (2015) Claudin-11 tight junctions in myelin are a barrier to diffusion and lack strong adhesive properties. Biophys J 109(7):1387–1397. https://doi.org/10.1016/j.bpj.2015.08.012

Devaux J, Gow A (2008) Tight junctions potentiate the insulative properties of small CNS myelinated axons. J Cell Biol 183:909–921. https://doi.org/10.1083/jcb.200808034

Dietrich RB, Bradley WG, Zaragoza EJ 4th, Otto RJ, Taira RK, Wilson GH, Kangarloo H (1988) MR evaluation of early myelination patterns in normal and developmentally delayed infants. Am J Roentgenol 150:889–896

Dietschy JM (2009) Central nervous system: cholesterol turnover, brain development and neurodegeneration. Biol Chem 390:287–293

Dimou L, Simon C, Kirchhoff F, Takebayashi H, Götz M (2008) Progeny of Olig2-expressing progenitors in the grey and white matter of the adult mouse cerebral cortex. J Neurosci 28: 10434–10442

Dimou L, Simons M (2017) Diversity of oligodendrocytes and their progenitors. Curr Opin Neurobiol 47:73–79. https://doi.org/10.1038/nature11314

Donaldson HH, Hoke GW (1905) On the areas of the axis cylinder and medullary sheath as seen in cross sections of the spinal nerves of vertebrates. J Comp Neurol 15:1–16

Duncan GJ, Manesh SB, Hilton BJ, Assinck P, Liu J, Moulson A, Plemel JR, Tetzlaff W (2018) Locomotor recovery following contusive spinal cord injury does not require oligodendrocyte remyelination. Nat Commun 9:3066. https://doi.org/10.1038/s41467-018-05473-1

Duncan GJ, Manesh SB, Hilton BJ, Assinck P, Plemel JR, Tetzlaff W (2020) The fate and function of oligodendrocyte progenitor cells after traumatic spinal cord injury. Glia 68:227–245

Duncan GJ, Simkins TJ, Emery B (2021) Neuron-oligodendrocyte interactions in the structure and integrity of axons. Front Cell Dev Biol 9:653101. https://doi.org/10.3389/fcell.2021.653101

Duncan ID, Radcliff AB (2016) Inherited and acquired disorders of myelin: the underlying myelin pathology. Exp Neurol 283:452–475

Eckhardt M (2008) The role and metabolism of sulfatide in the nervous system. Mol Neurobiol 37: 93–103. https://doi.org/10.1007/s12035-008-8022-3

Emery B, Lu QR (2015) Transcriptional and epigenetic regulation of oligodendrocyte development and myelination in the central nervous system. Cold Spring Harb Perspect Biol 7(9):a020461

Fancy SP, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, Sanai N, Franklin RJ, Rowitch DH (2009) Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. Genes Dev 23:1571–1585

Fancy SPJ, Harrington EP, Yuen TJ, Silbereis JC, Zhao C, Baranzini SE, Bruce CC, Otero JJ, Huang EJ, Nusse R, Franklin RJ (2011) Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. Nat Neurosci 14:1009–1016

Ferenczy MW, Marshall LJ, Nelson CDS, Atwood WJ, Nath A, Khalili K, Major EO (2012) Molecular biology, epidemiology, and pathogenesis of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev 2012:471–506

Fields RD (2008) White matter in learning, cognition and psychiatric disorders. Trends Neurosci 31:361–370. https://doi.org/10.1016/j.tins.2008.04.001

Fields RD (2010) Change in the brain's WM. Science 330:768–769. https://doi.org/10.1126/science.1199139

Fields RD (2015) A new mechanism of nervous system plasticity: activity-dependent myelination. Nat Rev Neurosci 16(12):756–767

Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, Rocca AM (2018) Multiple sclerosis. Nat Rev Dis Primers 4:1–27. https://doi.org/10.1038/s41572-018-0041-4

Filley CM, Fields RD (2016) White matter and cognition: making the connection. J Neurophysiol 116:2093–2104. https://doi.org/10.1152/jn.00221.2016

Ford MC, Alexandrova O, Cossell L, Stange-Marten A, Sinclair J, Kopp-Scheinpflug C, Pecka M, Attwell D, Grothe B (2015) Tuning of Ranvier node and internode properties in myelinated axons to adjust action potential timing. Nat Commun 6:1–14

Franklin RJ (2002) Why does remyelination fail in multiple sclerosis? Nat Rev Neurosci 3:705–714. https://doi.org/10.1038/nrn917

Franklin RJ (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9:839–855. https://doi.org/10.1038/nrn2480

Franklin RJ, Edgar JM, Smith KJ (2012) Neuroprotection and repair in multiple sclerosis. Nat Rev Neurol 8:624–634

Funfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Mobius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes CT, Frahm J, Goebbels S, Nave KA (2012) Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature 485:517–521

Gaesser JM, Fyffe-Maricich SL (2016) Intracellular signaling pathway regulation of myelination and remyelination in the CNS. Exp Neurol 283:501–511

Gallo V, Deneen B (2014) Glial development: the crossroads of regeneration and repair in the CNS. Neuron 83:283–308. https://doi.org/10.1016/j.neuron.2014.06.010

Galloway DA, Moore CS (2016) miRNAs as emerging regulators of oligodendrocyte development and differentiation. Front Cell Dev Biol 4:59

Garbern JY, Yool DA, Moore GJ, Wilds IB, Faulk MW, Klugmann M, Nave KA, Sistermans EA, van der Knaap MS, Bird TD, Shy ME, Kamholz JA, Griffiths IR (2002) Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation. Brain 125:551–561. https://doi.org/10.1093/brain/awf043

Gard AL, Burrell MR, Pfeiffer SE, Rudge JS, Williams WC (1995) Astroglial control of oligodendrocyte survival mediated by PDGF and leukemia inhibitory factor-like protein. Development 121:2187–2197

Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, Inema I, Miller SE, Bieri G, Zuchero JB, Barres BA, Woo PJ, Vogel H, Monje M (2014) Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. Science 344:1252304. https://doi.org/10.1126/science.1252304

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL (1999) Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 2:861–863

Goldenberg MM (2012) Multiple sclerosis review. PT 37(3):175–184

Gómez-Pinedo U, Sirerol-Piquer MS, Durán-Moreno M, García-Verdugo JM, Matias-Guiu J (2017) Alexander disease mutations produce cells with coexpression of glial fibrillary acidic protein and NG2 in neurosphere cultures and inhibit differentiation into mature oligodendrocytes. Front Neurol 8:255. https://doi.org/10.3389/fneur.2017.00255

Gruchot J, Weyers V, Göttle P, Förster M, Hartung HP, Küry P, Kremer D (2019) The molecular basis for remyelination failure in multiple sclerosis. Cell 8:825. https://doi.org/10.3390/cells8080825

Hagberg H, Peebles D, Mallard C (2002) Models of white matter injury: comparison of infectious, hypoxic-ischemic, and excitotoxic insults. Ment Retard Dev Disabil Res Rev 8:30–38

Harding B, Ellis D, Malcolm S (1995) A case of Pelizaeus-Merzbacher disease showing increased dosage of the proteolipid protein gene. Neuropathol Appl Neurobiol 21:111–115. https://doi.org/10.1111/j.1365-2990.1995.tb01036.x

Hart MN, Earle KM (1975) Haemorrhagic and perivenous encephalitis: a clinical-pathological review of 38 cases. J Neurol Neurosurg Psychiatry 38:585–591. https://doi.org/10.1136/jnnp.38.6.585

Hedden T, Schultz AP, Rieckmann A, Mormino EC, Johnson KA, Sperling RA, Buckner RL (2016) Multiple brain markers are linked to age-related variation in cognition. Cereb Cortex 26:1388–1400

Herwerth M, Kalluri SR, Srivastava R, Kleele T, Kenet S, Illes Z, Merkler D, Bennett JL, Misgeld T, Hemmer B (2016) In vivo imaging reveals rapid astrocyte depletion and axon damage in a model of neuromyelitis optica related pathology. Ann Neurol 79:794–805. https://doi.org/10.1002/ana.24630

Hildebrand C (1971) Ultrastructural and light-microscopic studies of the developing feline spinal cord white matter. I. the nodes of Ranvier. Acta Physiol Scand Suppl S364:81–107. https://doi.org/10.1111/j.1365-201X.1971.tb10979.x

Hildebrand C, Remahl S, Persson H, Bjartmar C (1993) Myelinated nerve fibres in the CNS. Prog Neurobiol 40:319–384

Höftberger R, Lassmann H (2017) Inflammatory demyelinating diseases of the central nervous system. Handb Clin Neurol 145:263–283. https://doi.org/10.1016/B978-0-12-802395-2.00019-5

Hollund IM, Olsen A, Skranes J, Brubakk AM, Håberg AK, Eikenes L, Evensen KA (2017) WM alterations and their associations with motor function in young adults born preterm with very low birth weight. Neuroimage Clin 17:241–250. https://doi.org/10.1016/j.nicl.2017.10.006

Hübner CA, Orth U, Senning A, Steglich C, Kohlschütter A, Korinthenberg R, Gal A (2005) Seventeen novel PLP1 mutations in patients with Pelizaeus-Merzbacher disease. Hum Mutat 25:321–322. https://doi.org/10.1002/humu.9314

Hughes EG, Kang SH, Fukaya M, Bergles DE (2013) Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nat Neurosci 16:668–676

Hughes EG, Orthmann-Murphy JL, Langseth AJ, Bergles DE (2018) Myelin remodeling through experience-dependent oligodendrogenesis in the adult somatosensory cortex. Nat Neurosci 21:696–706

Huxley AF, Stämpfli R (1949) Evidence for saltatory conduction in peripheral myelinated nerve fibres. J Physiol 108:315–339. https://doi.org/10.1113/jphysiol.1949.sp004335

Inder TE, Huppi PS (2000) In vivo studies of brain development by magnetic resonance techniques. Ment Retard Dev Disabil Res Rev 6:59–67

Jeffries MA, Urbanek K, Torres L, Wendell SG, Rubio ME, Fyffe-Maricich SL (2016) ERK1/2 activation in pre-existing oligodendrocytes of adult mice drives new myelin synthesis and enhanced CNS function. J Neurosci 36:9186–9200

Jin J, Peng Q, Hou Z, Jiang M, Wang X, Langseth AJ, Tao M, Barker PB, Mori S, Bergles DE, Ross CA (2015) Early white matter abnormalities, progressive brain pathology and motor deficits in a novel knock-in mouse model of Huntington's disease. Hum Mol Genet 24:2508–2527

Juurlink BHJ, Thorburne SK, Hertz L (1998) Peroxide-scavenging deficit underlies oligodendrocyte susceptibility to oxidative stress. Glia 22:371–378

Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE (2010) NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron 68:668–681

Karim SA, Barrie JA, McCulloch MC, Montague P, Edgar JM, Kirkham D, Anderson TJ, Nave KA, Griffiths IR, McLaughlin M (2007) PLP overexpression perturbs myelin protein composition and myelination in a mouse model of Pelizaeus-Merzbacher disease. Glia 55:341–351. https://doi.org/10.1002/glia.20465

Keirstead HS, Blakemore WF (1999) The role of oligodendrocytes and oligodendrocyte progenitors in CNS remyelination. Adv Exp Med Biol 468:183–197

Kinney HC, Brody BA, Kloman AS, Gilles FH (1988) Sequence of central nervous system myelination in human infancy. II. Patterns of myelination in autopsied infants. J Neuropathol Exp Neurol 47:217–234. https://doi.org/10.1097/00005072-198805000-00003

Klingseisen A, Lyons DA (2018) Axonal regulation of central nervous system myelination: structure and function. Neuroscientist 24(1):7–21

Knickmeyer RC, Styner M, Short SJ, Lubach GR, Kang C, Hamer R, Coe CL, Gilmore JH (2010) Maturational trajectories of cortical brain development through the pubertal transition: unique species and sex differences in the monkey revealed through structural magnetic resonance imaging. Cereb Cortex 20(5):1053–1063

Kobayashi Y, Tohyama J, Akiyama T, Magara S, Kawashima H, Akasaka N, Nakashima M, Saitsu H, Matsumoto N (2016) Severe leukoencephalopathy with cortical involvement and peripheral neuropathy due to FOLR1 deficiency. Brain Dev 39:266–270

Kohama SG, Rosene DL, Sherman LS (2012) Age-related changes in human and non-human primate white matter: from myelination disturbances to cognitive decline. Age 34:1093–1110

Kohlschütter A (2013) Lysosomal leukodystrophies: Krabbe disease and metachromatic leukodystrophy. Handb Clin Neurol 113:1611–1618. https://doi.org/10.1016/B978-0-444-59565-2.00029-0

Kotter MR, Li WW, Zhao C, Franklin RJ (2006) Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. J Neurosci 26:328–332

Koudelka S, Voas MG, Almeida RG, Baraban M, Soetaert J, Meyer MP, Talbot WS, Lyons DA (2016) Individual neuronal subtypes exhibit diversity in CNS myelination mediated by synaptic vesicle release. Curr Biol 26:1447–1455

Kuhlmann T, Miron V, Cuo Q, Wegner C, Antel J, Brück W, Kuhlmann T (2008) Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 131(7):1749–1758

Kuhn S, Gritti L, Crooks D, Dombrowski Y (2019) Oligodendrocyte in development, myelin generation and beyond. Cell 8:1424

Kumar R, Chavez AS, Macey PM, Woo MA, Harper RM (2013) Brain axial and radial diffusivity changes with age and gender in healthy adults. Brain Res 1512:22–36. https://doi.org/10.1016/j.brainres.2013.03.028

Lassmann H (2003) Hypoxia-like tissue injury as a component of multiple sclerosis lesions. J Neurol Sci 206:187–191

Lebel C, Gee M, Camicioli R, Wieler M, Martin W, Beaulieu C (2012) Diffusion tensor imaging of WM tract evolution over the lifespan. Neuroimage 60:340–352. https://doi.org/10.1016/j.neuroimage.2011.11.094

Lebel C, Treit S, Beaulieu C (2019) A review of diffusion MRI of typical WM development from early childhood to young adulthood. NMR Biomed 32(4):e3778. https://doi.org/10.1002/nbm.3778

Lee JY, Petratos S (2016) Thyroid hormone signaling in oligodendrocytes: from extracellular transport to intracellular signal. Mol Neurobiol 53:6568–6583

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ, Rothstein JD (2012) Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature 487:443–448. https://doi.org/10.1038/nature11314

Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, Nakashima I, Weinshenker BG (2004) A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 364:2106–2112. https://doi.org/10.1016/S0140-6736(04)17551-X

Liu J, Dietz K, DeLoyht JM, Pedre X, Kelkar D, Kaur J, Vialou V, Lobo MK, Dietz DM, Nestler EJ, Dupree J (2012) Impaired adult myelination in the prefrontal cortex of socially isolated mice. Nat Neurosci 15:1621–1623

Lundgaard I, Luzhynskaya A, Stockley JH, Wang Z, Evans KA, Swire M, Volbracht K, Gautier HO, Franklin RJ, Attwell D, Káradóttir RT (2013) Neuregulin and BDNF induce a switch to NMDA receptor-dependent myelination by oligodendrocytes. PLoS Biol 11:e1001743. https://doi.org/10.1371/journal.pbio.1001743

Macchi M, Magalon K, Zimmer C, Peeva E, El Waly B, Brousse B, Jaekel S, Grobe K, Kiefer F, Williams A, Cayre M (2020) Mature oligodendrocytes bordering lesions limit demyelination and favor myelin repair via heparan sulfate production. eLife 9:e51735. https://doi.org/10.7554/eLife.51735

Mader S, Gredler V, Schanda K, Rostasy K, Dujmovic I, Pfaller K, Lutterotti A, Jarius S, Di Pauli F, Kuenz B, Ehling R (2011) Complement activating antibodies to myelin oligodendrocyte glycoprotein in neuromyelitis optica and related disorders. J Neuroinflammation 8:184

Magnus T, Carmen J, Deleon J, Xue H, Pardo AC, Lepore AC, Mattson MP, Rao MS, Maragakis NJ (2008) Adult glial precursor proliferation in mutant SOD1G93A mice. Glia 56(2):200–208

Major EO (2010) Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies. Annu Rev Med 61:35–47. https://doi.org/10.1146/annurev.med.080708.082655

Makinodan M, Rosen KM, Ito S, Corfas G (2012) A critical period for social experience–dependent oligodendrocyte maturation and myelination. Science 337:1357–1360

Matute C, Sánchez-Gómez MV, Martínez-Millán L, Miledi R (1997) Glutamate receptor-mediated toxicity in optic nerve oligodendrocytes. Proc Natl Acad Sci U S A 94:8830–8835

Matute C, Torre I, Pérez-Cerdá F, Pérez-Samartín A, Alberdi E, Etxebarria E, Arranz AM, Ravid R, Rodríguez-Antigüedad A, Sánchez-Gómez M, Domercq M (2007) P2X 7 receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 27:9525–9533

Matveeva O, Bogie JFJ, Hendriks JJA, Linker RA, Haghikia A, Kleinewietfeld M (2018) Western lifestyle and immunopathology of multiple sclerosis. Ann N Y Acad Sci 1417:71–86. https://doi.org/10.1111/nyas.13583

McAleese KE, Walker L, Graham S, Moya EL, Johnson M, Erskine D, Colloby SJ, Dey M, Martin-Ruiz C, Taylor JP, Thomas AJ (2017) Parietal white matter lesions in Alzheimer's disease are associated with cortical neurodegenerative pathology, but not with small vessel disease. Acta Neuropathol 134:459–473

McKenzie IA, Ohayon D, Li H, de Faria JP, Emery B, Tohyama K, Richardson WD (2014) Motor skill learning requires active central myelination. Science 346:318–322. https://doi.org/10.1126/science.1254960

McKinnon RD, Waldron S, Kiel ME (2005) PDGFα-receptor signal strength controls an RTK rheostat that integrates phosphoinositol 3-kinase and phospholipase C pathways during oligodendrocyte maturation. J Neurosci 25:3499–3508

Mecha M, Carrillo-Salinas FJ, Mestre L, Feliú A, Guaza C (2013) Viral models of multiple sclerosis: neurodegeneration and demyelination in mice infected with Theiler's virus. Prog Neurobiol 101-102:46–64. https://doi.org/10.1016/j.pneurobio.2012.11.003

Mei F, Lehmann-Horn K, Shen YA, Rankin KA, Stebbins KJ, Lorrain DS, Pekarek K, Sagan SA, Xiao L, Teuscher C, von Büdingen HC (2016) Accelerated remyelination during inflammatory demyelination prevents axonal loss and improves functional recovery. Elife 5:e18246

Menichella DM, Goodenough DA, Sirkowski E, Scherer SS, Paul DL (2003) Connexins are critical for normal myelination in the CNS. J Neurosci 23:5963–5973

Messing A, Head MW, Galles K, Galbreath E, Goldman JE, Brenner M (1998) Fatal encephalopathy with astrocyte inclusions in GFAP transgenic mice. Am J Pathol 152:391–398

Mierzwa AJ, Marion CM, Sullivan GM, McDaniel DP, Armstrong RC (2015) Components of myelin damage and repair in the progression of white matter pathology after mild traumatic brain injury. J Neuropathol Exp Neurol 74:218–232

Miller DJ, Duka T, Stimpson CD, Schapiro SJ, Baze WB, McArthur MJ, Fobbs AJ, Sousa AM, Sestan N, Wildman DE, Lipovich L, Kuzawa CW, Hof PR, Sherwood CC (2012) Prolonged myelination in human neocortical evolution. Proc Natl Acad Sci U S A 109:16480–16485

Miller SD, Olson JK, Croxford JL (2001) Multiple pathways to induction of virus-induced autoimmune demyelination: lessons from Theiler's virus infection. J Autoimmun 16:219–227. https://doi.org/10.1006/jaut.2000.0489

Misu T, Fujihara K, Kakita A, Konno H, Nakamura M, Watanabe S, Takahashi T, Nakashima I, Takahashi H, Itoyama Y (2007) Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. Brain 130(Pt 5):1224–1234

Miyata S, Hattori T, Shimizu S, Ito A, Tohyama M (2015) Disturbance of oligodendrocyte function plays a key role in the pathogenesis of schizophrenia and major depressive disorder. Biomed Res Int 2015:492367

Monje M (2018) Myelin plasticity and nervous system function. Annu Rev Neurosci 41:61–76. https://doi.org/10.1146/annurev-neuro-080317-061853

Montague P, McCallion AS, Davies RW, Griffiths IR (2006) Myelin-associated oligodendrocytic basic protein: a family of abundant CNS myelin protein in search of a function. Dev Neurosci 28:479–487. https://doi.org/10.1159/000095110

Morell P, Jurevics H (1996) Origin of cholesterol in myelin. Neurochem Res 21:463–470

Mount CW, Monje M (2017) Wrapped to adapt: experience-dependent myelination. Neuron 95: 743–756. https://doi.org/10.1016/j.neuron.2017.07.009

Nakagawa H, Iwasaki S, Kichikawa K, Fukusumi A, Taoka T, Ohishi H, Uchida H (1998) Normal myelination of anatomic nerve fiber bundles: MR analysis. Am J Neuroradiol 19:1129–1136

Nave KA (2010) Myelination and the trophic support of long axons. Nat Rev Neurosci 11:275–283. https://doi.org/10.1038/nrn2797

Nave KA, Werner HB (2014) Myelination of the nervous system: mechanisms and functions. Annu Rev Cell Dev Biol 30:503–533. https://doi.org/10.1146/annurev-cellbio-100913-013101

Noble M, Murray K, Stroobant P, Waterfield MD, Riddle P (1988) Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. Nature 333:560–562. https://doi.org/10.1038/333560a0

Norton WT, Poduslo SE (1973) Myelination in rat brain: changes in myelin composition during brain maturation. J Neurochem 21:759–773

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG (2000) Multiple sclerosis. N Engl J Med 343:938–952. https://doi.org/10.1056/NEJM200009283431307

Ohlhauser L, Parker AF, Smart CM, Gawryluk JR (2018) WM and its relationship with cognition in subjective cognitive decline. Alzheimers Dement (Amst) 11:28–35

Olabarria M, Goldman JE (2017) Disorders of astrocytes: Alexander disease as a model. Annu Rev Pathol 12:131–152. https://doi.org/10.1146/annurev-pathol-052016-100218

Orentas DM, Hayes JE, Dyer KL, Miller RH (1999) Sonic hedgehog signaling is required during the appearance of spinal cord oligodendrocyte precursors. Development 126:2419–2429

Oztürk AH, Tasçioglu B, Aktekin M, Kurtoglu Z, Erden I (2002) Morphometric comparison of the human corpus callosum in professional musicians and non-musicians by using in vivo magnetic resonance imaging. J Neuroradiol 29:29–34

Paloneva J, Autti T, Raininko R, Partanen J, Salonen O, Puranen M, Hakola P, Haltia M (2001) CNS manifestations of Nasu-Hakola disease: a frontal dementia with bone cysts. Neurology 56: 1552–1558. https://doi.org/10.1212/WNL.56.11.1552

Paloneva J, Kestilä M, Wu J, Salminen A, Böhling T, Ruotsalainen V, Hakola P, Bakker ABH, Phillips JH, Pekkarinen P, Lanier LL, Timonen T, Peltonen L (2000) Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. Nat Genet 25: 357–361. https://doi.org/10.1038/77153

Panagiotakos G, Alshamy G, Chan B, Abrams R, Greenberg E, Saxena A, Bradbury M, Edgar M, Gutin P, Tabar V (2007) Long-term impact of radiation on the stem cell and oligodendrocyte precursors in the brain. PLoS One 2:e588

Parratt JD, Prineas JW (2010) Neuromyelitis optica: a demyelinating disease characterized by acute destruction and regeneration of perivascular astrocytes. Mult Scler 16:1156–1172. https://doi.org/10.1177/1352458510382324

Patro N, Naik AA, Patro IK (2019) Developmental changes in oligodendrocyte genesis, myelination, and associated behavioral dysfunction in a rat model of intra-generational protein malnutrition. Mol Neurobiol 56:595–610

Pekny M, Pekna M, Messing A, Steinhäuser C, Lee JM, Parpura V, Hol EM, Sofroniew MV, Verkhratsky A (2016) Astrocytes: a central element in neurological diseases. Acta Neuropathol 131:323–345. https://doi.org/10.1007/s00401-015-1513-1

Peters A (2002) The effects of normal aging on myelin and nerve fibers: a review. J Neurocytol 31: 581–593. https://doi.org/10.1023/A:1025731309829

Philips T, Rothstein JD (2017) Oligodendroglia: metabolic supporters of neurons. Clin Investig 127(9):3271–3280. https://doi.org/10.1172/JCI90610

Plemel JR, Keough MB, Duncan GJ, Sparling JS, Yong VW, Stys PK, Tetzlaff W (2014) Remyelination after spinal cord injury: is it a target for repair? Prog Neurobiol 117:54–72

Popescu BFG, Lucchinetti CF (2011) Pathology of demyelinating diseases. Annu Rev Pathol Mech Dis 7:185–217. https://doi.org/10.1146/annurev-pathol-011811-132443

Pouwels PJ, Vanderver A, Bernard G, Wolf NI, Dreha-Kulczewksi SF, Deoni SC, Bertini E, Kohlschutter A, Richardson W, French-Constant C, Kohler W, Rowitch D, Barkovich AJ (2014) Hypomyelinating leukodystrophies: translational research progress and prospects. Ann Neurol 76:5–19

Prado EL, Dewey KG (2014) Nutrition and brain development in early life. Nutr Rev 72:267–284

Pronker MF, Lemstra S, Snijder J, Heck AJR, Thies-Weesie DME, Pasterkamp RJ, Janssen BJC (2016) Structural basis of myelin-associated glycoprotein adhesion and signalling. Nat Commun 7:13584

Rademakers R, Baker M, Nicholson AM, Rutherford NJ, Finch N, Soto-Ortolaza A, Lash J, Wider C, Wojtas A, DeJesus-Hernandez M, Adamson J, Kouri N, Sundal C, Shuster EA, Aasly J, MacKenzie J, Roeber S, Kretzschmar HA, Boeve BF, Knopman DS, Petersen RC, Cairns NJ, Ghetti B, Spina S, Garbern J, Tselis AC, Uitti R, Das P, Van Gerpen JA, Meschia JF, Levy S, Broderick DF, Graff-Radford N, Ross OA, Miller BB, Swerdlow RH, Dickson DW, Wszolek ZK (2012) Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. Nat Genet 44:200–205. https://doi.org/10.1038/ng.1027

Rasband MN, Park EW, Vanderah TW, Lai J, Porreca F, Trimmer JS (2001) Distinct potassium channels on pain-sensing neurons. Proc Natl Acad Sci U S A 98:13373–13378. https://doi.org/10.1073/pnas.231376298

Reich DS, Lucchinetti CF, Calabresi PA (2018) Multiple sclerosis. N Engl J Med 378:169–180. https://doi.org/10.1056/NEJMra1401483

Remahl S, Hildebrand C (1982) Changing relation between onset of myelination and axon diameter range in developing feline white matter. J Neurol Sci 54:33–45

Richardson EPJ (1961) Progressive multifocal leukoencephalopathy. N Engl J Med 265:815–823

Robinson JL, Suh E, Wood EM, Lee EB, Coslett HB, Raible K, Lee VMY, Trojanowski JQ, Van Deerlin VM (2015) Common neuropathological features underlie distinct clinical presentations

in three siblings with hereditary diffuse leukoencephalopathy with spheroids caused by CSF1R p.Arg782His. Acta Neuropathol Commun 3:42. https://doi.org/10.1186/s40478-015-0219-x

Rogister B, Ben-Hur T, Dubois-Dalcq M (1999) From neural stem cells to myelinating oligodendrocytes. Mol Cell Neurosci 14:287–300

Rone MB, Cui QL, Fang J, Wang LC, Zhang J, Khan D, Bedard M, Almazan G, Ludwin SK, Jones R, Kennedy TE (2016) Oligodendrogliopathy in multiple sclerosis: low glycolytic metabolic rate promotes oligodendrocyte survival. J Neurosci 36:4698–4707

Rosenberg SS, Kelland EE, Tokar E, La Torre De AR, Chan JR (2008) The geometric and spatial constraints of the microenvironment induce oligodendrocyte differentiation. Proc Natl Acad Sci U S A 105:14662–14667

Ruckh JM, Zhao JW, Shadrach JL, Van Wijngaarden P, Rao TN, Wagers AJ, Franklin RJM (2012) Rejuvenation of regeneration in the aging central nervous system. Cell Stem Cell 10:96–103

Saab AS, Tzvetavona ID, Trevisiol A, Baltan S, Dibaj P, Kusch K, Möbius W, Goetze B, Jahn HM, Huang W, Steffens H (2016) Oligodendroglial NMDA receptors regulate glucose import and axonal energy metabolism. Neuron 91:119–132

Saher G, Brugger B, Lappe-Siefke C, Mobius W, Ryu-ichi T, Wehr MC, Wieland F, Shun Ishibashi S, Klaus-Armin Nave KA (2005) High cholesterol level is essential for myelin membrane growth. Nat Neurosci 8(4):468–475

Saher G, Stumpf SK (2015) Cholesterol in myelin biogenesis and hypomyelinating disorders. Biochim Biophys Acta 1851(8):1083–1094

Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, Dilthey A, Su Z, Freeman C, Hunt SE, Edkins S (2011) International multiple sclerosis genetics consortium; Wellcome Trust case control consortium 2 genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 476:214–219. https://doi.org/10.1038/nature10251

Schmitt S, Castelvetri LC, Simons M (2015) Metabolism and functions of lipids in myelin. BiochimBiophys Acta 1851:999–1005. https://doi.org/10.1016/j.bbalip.2014.12.016

Scolding N (2014) Acute disseminated encephalomyelitis and other inflammatory demyelinating variants, 1st edn. Elsevier, Amsterdam

Semple BD, Blomgren K, Gimlin K, Ferriero M, Noble-Haeusslein LJ (2013) Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol 2013:1–16

Seth P, Diaz F, Tao-Cheng JH, Major EO (2004) JC virus induces non apoptotic cell death of human central nervous system progenitor cell-derived astrocytes. J Virol 78:4884–4891. https://doi.org/10.1128/JVI.78.9.4884-4891.2004

Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN (2008) Neurodevelopmental trajectories of the human cerebral cortex. J Neurosci 28:3586–3594

Silveira AH, Patil N, Brickner MA, Parr AM (2021) Strategies for oligodendrocyte and myelin repair in traumatic CNS injury. Front Cell Neurosci 14:619707. https://doi.org/10.3389/fncel.2020.619707

Sim FJ, Zhao C, Penderis J, Franklin RJM (2002) The age related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. J Neurosci 22:2451–2459

Sima AA, Pierson CR, Woltjer RL, Hobson GM, Golden JA, Kupsky WJ, Schauer GM, Bird TD, Skoff RP, Garbern JY (2009) Neuronal loss in Pelizaeus-Merzbacher disease differs in various mutations of the proteolipid protein 1. Acta Neuropathol 118:531–539. https://doi.org/10.1007/s00401-009-0562-8

Simons M, Kramer EM, Macchi P, Rathke-Hartlieb S, Trotter J, Nave KA, Schulz JB (2002) Overexpression of the myelin proteolipid protein leads to accumulation of cholesterol and proteolipid protein in endosomes/lysosomes: implications for Pelizaeus-Merzbacher disease. J Cell Biol 157:327–336. https://doi.org/10.1083/jcb.200110138

Simons M, Krämer EM, Thiele C, Stoffel W, Trotter J (2000) Assembly of myelin by association of proteolipid protein with cholesterol- and galactosylceramide-rich membrane domains. J Cell Biol 151:143–154

Singh K, Patro N, Pradeepa M, Patro I (2017) Neonatal lipopolysaccharide infection causes demyelination and behavioral deficits in adult and senile rat brain. Ann Neurosci 24:146–154

Sloane JA, Batt C, Ma Y, Harris ZM, Trapp B, Vartanian T (2010) Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. Proc Natl Acad Sci U S A 107: 11555–11560

Snaidero N, Möbius W, Czopka T, Hekking LH, Mathisen C, Verkleij D, Goebbels S, Edgar J, Merkler D, Lyons DA, Nave KA (2014) Myelin membrane wrapping of CNS axons by PI(3,4,5) P3-dependent polarized growth at the inner tongue. Cell 156:277–290

Snaidero N, Simons M (2014) Myelination at a glance. J Cell Sci 127:2999–3004

Snaidero N, Velte C, Myllykoski M, Raasakka A, Ignatev A, Werner HB, Erwig MS, Möbius W, Kursula P, Nave KA, Simons M (2017) Antagonistic functions of MBP and CNP establish cytosolic channels in CNS myelin. Cell Rep 18(2):314–323

Sommer I, Schachner M (1981) Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. Dev Biol 83:311–327

Sosunov AA, Guilfoyle E, Wu X, McKhann GM 2nd, Goldman JE (2013) Phenotypic conversions of "protoplasmic" to "reactive" astrocytes in Alexander disease. J Neurosci 33:7439–7450

Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW (2003) Mapping cortical change across the human life span. Nat Neurosci 6:309–315. https://doi.org/10.1038/nn1008

Stadelmann C, Timmler S, Barrantes-Freer A, Simons M (2019) Myelin in the central nervous system: structure, function and pathology. Physiol Rev 99:1381–1431

Steele CJ, Bailey JA, Zatorre RJ, Penhune VB (2013) Early musical training and white matter plasticity in the corpus callosum: evidence for a sensitive period. J Neurosci 33:1282–1290. https://doi.org/10.1523/JNEUROSCI.3578-12.2013

Sullivan EV, Adalsteinsson E, Hedehus M, Ju C, Moseley M, Lim KO, Pfefferbaum A (2001) Equivalent disruption of regional white matter microstructure in ageing healthy men and women. Neuroreport 12:99–104

Tasker RC (2006) Changes in white matter late after severe traumatic brain injury in childhood. Dev Neurosci 28:302–308

Tomassy GS, Lori Bowe Dershowitz LB, Arlotta P (2016) Diversity matters: a revised guide to myelination. Trends Cell Biol 26(2):135–147. https://doi.org/10.1016/j.tcb.2015.09.002

Tongatta R, Miller RH (2016) Contribution of the oligodendrocyte lineage to CNS repair and neurodegenerative pathology. Neuropharmacology 110(B):539–547

Torii T, Miyamoto Y, Yamauchi J, Tanoue A (2014) Pelizaeus-Merzbacher disease: cellular pathogenesis and pharmacologic therapy. Pediatr Int 56:659–666. https://doi.org/10.1111/ped.12450

Tradtrantip L, Yao X, Su T, Smith AJ, Verkman AS (2017) Bystander mechanism for complement initiated early oligodendrocyte injury in neuromyelitis optica. Acta Neuropathol 134:35–44. https://doi.org/10.1007/s00401-017-1734-6

Traka M, Podojil JR, McCarthy DP, Miller SD, Popko B (2016) Oligodendrocyte death results in immune-mediated CNS demyelination. Nat Neurosci 19:65–74

Trapp BD, Nave KA (2008) Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci 31:247–269. https://doi.org/10.1146/annurev.neuro.30.051606.094313

Tsutsui S, Stys PK (2013) Metabolic injury to axons and myelin. Exp Neurol 246:26–34

van der Knaap MS, Bugiani M (2017) Leukodystrophies: a proposed classification system based on pathological changes and pathogenetic mechanisms. Acta Neuropathol 134:351–382

Vartanian T, Fischbach G, Miller R (1999) Failure of spinal cord oligodendrocyte development in mice lacking neuregulin. Proc Natl Acad Sci U S A 96:731–735

Volpe JJ (2000) Overview: normal and abnormal human brain development. Ment Retard Dev Disabil Res Rev 6:1–5

Wake H, Ortiz FC, Woo DH, Lee PR, Angulo MC, Fields RD (2015) Nonsynaptic junctions on myelinating glia promote preferential myelination of electrically active axons. Nat Commun 6: 7844

Weinshenker BG, Wingerchuk DM (2017) Neuromyelitis spectrum disorders. Mayo Clin Proc 92: 663–679. https://doi.org/10.1016/j.mayocp.2016.12.014

Wiggins RC (1986) Myelination: a critical stage of development. Neurotoxicology 7:103–120

Wiley CA, Grafe M, Kennedy C, Nelson JA (1988) Human immunodeficiency virus (HIV) and JC virus in acquired immune deficiency syndrome (AIDS) patients with progressive multifocal leukoencephalopathy. Acta Neuropathol 76:338–346

Williamson JM, Lyons DA (2018) Myelin dynamics throughout life: an ever-changing landscape? Front Cell Neurosci 12:424. https://doi.org/10.3389/fncel.2018.00424

Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, de Seze J, Fujihara K, Greenberg B, Jacob A, Jarius S, Lana-Peixoto M, Levy M, Simon JH, Tenembaum S, Traboulsee AL, Waters P, Wellik KE, Weinshenker BG (2015) International panel for NMO diagnosis. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 85:177–189. https://doi.org/10.1212/WNL.0000000000001729

Wlodarczyk A, Holtman IR, Krueger M, Yogev N, Bruttger J, Khorooshi R, Benmamar-Badel A, de Boer-Bergsma JJ, Martin NA, Karram K, Kramer I (2017) A novel microglial subset plays a key role in myelinogenesis in developing brain. EMBO J 36:3292–3308

Wolf NI, Ffrench-Constant C, van der Knaap MS (2021) Hypomyelinating leukodystrophies-unravelling myelin biology. Nat Rev Neurol 17(2):88–103

Wollebo HS, White MK, Gordon J, Berger JR, Khalili K (2015) Persistence and pathogenesis of the neurotropic polyomavirus JC. Ann Neurol 77:560–570. https://doi.org/10.1002/ana.24371

Wrzos C, Winkler A, Metz I, Kayser DM, Thal DR, Wegner C, Brück W, Nessler S, Bennett JL, Stadelmann C (2014) Early loss of oligodendrocytes in human and experimental neuromyelitis optica lesions. Acta Neuropathol 127:523–538. https://doi.org/10.1007/s00401-013-1220-8

Xiao L, Ohayon D, McKenzie IA, Sinclair-Wilson A, Wright JL, Fudge AD, Emery B, Li H, Richardson WD (2016) Rapid production of new oligodendrocytes is required in the earliest stages of motor-skill learning. Nat Neurosci 19:1210–1217. https://doi.org/10.1038/nn.4351

Yakovlev PI, Lecours A (1967) The myelogenetic cycles of regional maturation of the brain. In: Minkowski A (ed) Regional development of the brain in early life. Blackwell Science, Oxford, pp 3–70

Yeung MS, Zdunek S, Bergmann O, Bernard S, Salehpour M, Alkass K, Perl S, Tisdale J, Possnert G, Brundin L, Druid H (2014) Dynamics of oligodendrocyte generation and myelination in the human brain. Cell 159:766–774

Young KM, Psachoulia K, Tripathi RB, Dunn S-J, Cossell L, Attwell D, Tohyama K, Richardson WD (2013) Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodelling. Neuron 77:873–885

Young NP, Weinshenker BG, Parisi JE, Scheithauer B, Giannini C, Roemer SF, Thomsen KM, Mandrekar JN, Erickson BJ, Lucchinetti CF (2010) Perivenous demyelination: association with clinically defined acute disseminated encephalomyelitis and comparison with pathologically confirmed multiple sclerosis. Brain 133:333–348. https://doi.org/10.1093/brain/awp321

Zuchero JB, Fu MM, Sloan SA, Ibrahim A, Olson A, Zaremba A, Dugas JC, Wienbar S, Caprariello AV, Kantor C, Leonoudakis D (2015) CNS myelin wrapping is driven by actin disassembly. Dev Cell 34(2):152–167

Oligodendroglial-Astroglial Cell-Cell Communication in the Central Nervous System

Rahul Basu and Jayasri Das Sarma

Abstracts

The central nervous system (CNS) is mainly comprised of neurons and glial cells, namely, astrocytes, oligodendrocytes, and microglia. Astrocytes control metabolic coupling and small molecule homeostasis among the neurons and other glial cells. The metabolic coupling is mainly facilitated by gap junctions (GJs), consisted of connexin (Cx) proteins. Astrocytes are functionally connected through oligodendroglial-astroglial cell-cell communication in the central nervous system with all other neuroglial cells that provides metabolic support and homeostasis to maintain CNS health. Glial cells, specifically the astrocytes and oligodendrocytes, form several hemichannels and GJICs that help in ionic and small molecule exchange (<1KD). In addition to that, several soluble factors and signaling molecules are controlled by the functional GJICs. One of the major functions of the astrocytic GJs is to maintain ionic homeostasis during neuronal activity and propagation of action potential, where GJs provide a direct pathway for electrical and metabolic signaling between CNS cells. In addition, GJs have a pivotal role in maintaining myelin function and in its maintenance. In this chapter, we discuss how astrocytes and other glial cells are connected through GJICs. These GJICs control the formation and maintenance of myelin, which results in the proper functioning of the central and peripheral nervous systems (CNS and PNS). Specifically, the functions of individual GJPs are discussed with an emphasis on astrocytic/astrocytic Cx43/Cx43 homotypic and astrocytic/oligodendrocytic Cx43/Cx47 heterotypic channels having a major role in myelination and its maintenance.

R. Basu · J. D. Sarma (✉)
Department of Biological Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur, India
e-mail: dassarmaj@iiserkol.ac.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_4

Keywords

Astrocyte · Oligodendrocyte · Gap junction · oligodendroglial-astroglial cell-cell communication in the central nervous system · Connexins · Connexin43 · Connexin47 · Metabolic coupling

1 Introduction

A unique adaptation of glia in vertebrates is the multilamellar myelin sheath wrapping around the long axon formed by the deposition of proteolipid protein (PLP) and myelin-associated glycoprotein (MAG) and myelin basic protein (MBP). Myelin sheath is formed by the oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). Compaction of myelin surrounding the axon is essential as it builds an insulating layer with regular gaps between long axon segments termed as nodes of Ranvier which leads to intimate cell-cell interactions in the nervous system. The compaction of myelin depends on the abundant expression of unique lipid with integral myelin-limited protein PLP and MAG. While PLP and MAG provide structural support within the myelin membranes, MBP is important for creating scaffold for attachment to lipids as well as to diverse membrane proteins including intercellular channel forming gap junction (GJ) proteins, ionic channels, transporters, as well as cytoskeletal proteins and signaling molecules. Signaling through myelination within the myelinating glial cell and axon has been shown to play a crucial role for axonal integrity and survival. Myelin abnormalities cause a number of neurological diseases with demyelinating and dysmyelination neuropathies. These neuropathies are caused either due to malformed and defective myelin sheath arise from hereditary mutations typically, known as leukodystrophies or dysmyelination or chronic spontaneous loss of myelin known as demyelination or due to genetic predisposition, certain infections, some CNS/PNS autoimmune disorders and exposure to toxic chemicals. A large number of demyelination and dysmyelination pathologies have been concomitant to alternation of oligodendroglial-astroglial cell-cell communication in the central nervous system with adjacent glial cells, mainly astrocytes, connecting their cytoplasm to other neuroglial cells. Astrocytes maintain the neuronal homeostasis and contribute to metabolic coupling among neural cells, electrical coupling, and spatial buffering. Metabolic coupling between neuroglial cells largely occurs through the panglial homotypic and heterotypic gap junction channels (GJCs). GJCs allow the exchange of ions and small metabolites up to 1 kDa. Alterations in GJC forming protein connexins (Cxs) present in the myelinating cells and astrocytes may promote the demyelinating and dysmyelinating diseases. Astrocytes, the major glial cell type in the CNS, may be indirectly associated with the myelin protein formation and myelination process mainly via GJIC-mediated small molecule signaling required for oligodendrocyte differentiation and their metabolic activity . In this chapter, we will discuss the evidence that supports a role for Cxs present in both astrocytes and oligodendrocytes which takes part in the process of myelination, dysmyelination, and demyelination. The paradigm shifts in our understanding of

panglial heterocellular gap junction communication through Cx protein between astrocytes and oligodendrocytes in the CNS might be important to elucidate the mechanism of nervous system diseases and disorders and helpful to design potential therapeutic interventions.

2 The Myelin Sheath

The myelin sheath is a proteolipid enwrapping around the neurons in CNS and PNS. The myelin sheath helps in the salutatory conduction of action potentials between neurons, forms an insulating layer around the neurons that help in metabolic coupling, prevents ion leakage, and prevents axonal injury. The myelin sheath is mainly consisted of lipids like glycosphingolipids (GalC), cholesterol, sulfatides and proteins like PLP, its spliced isoform DM20, MAG, MBP in CNS. PNS myelin also contains myelin protein zero (MPZ), peripheral myelin protein 2 (PMP2), peripheral myelin protein 22 (PMP 22) and stathmin. Several genetic dysmyelinating diseases and spontaneous demyelinating diseases have significant impact on the functioning of the nervous system due to abnormal formation or degeneration of myelin sheath. Genetic mutations in myelin proteins or myelin-/nervous system-associated cell junction proteins can give rise to several dysmyelinating diseases, whereas demyelinating diseases are mainly spontaneous and etiologically related to either autoimmunity caused by myelin-reactive T-cells' migration into the CNS or viral-induced innate immune inflammation resulting in chronic progressive demyelination or toxin-induced direct damage to the white matter myelin. Formation and maintenance of myelin are tightly regulated by signaling between the glial cells and glial cells to neurons or vice versa. For example, neuronal signaling-dependent leukemia inhibitory factor (LIF) production by astrocytes helps in MOG synthesis by oligodendrocytes. On the other hand, metabolic coupling between astrocytes and astrocytes to oligodendrocytes via GJs and other signaling molecules help in the maintenance of myelin proteins and neuronal health. Thus, in this chapter, we will mainly discuss about the panglial (astrocyte-to-astrocyte or astrocyte-to-oligodendrocyte) communications in the perspective of maintaining myelin sheath structure and function, which, in turn, maintain the neurons to confer proper functioning of the nervous system.

3 Panglial Astro-Oligo Metabolic Coupling and Maintenance of CNS Homeostasis

Astrocytes have been previously considered to be a group of non-excitable "glueing" cells of the brain and are believed to mainly tether the functional neuronal cells together. In mammalian brain, astrocytes are present most abundantly in numbers and count to be at least equal to, or exceeding, those of the neurons. In the 1970s, Stephen Kuffler proposed the role of glial cells, which initiated a novel field of research on glial biology to investigate mechanisms of neuron-glia interactions

Table 1 Panglial GJs and their heterotypic coupling partners

Gap junction protein	Heterotypic coupling partner	Localization
Cx43	Cx43 and Cx47	Gray and white matter
Cx30	Cx30 and Cx32	Mainly gray matter
Cx47	Cx43	Mainly white matter
Cx32	Cx32 and Cx30	Mainly gray matter

(Kuffler 1967). Decades later, it was found that astrocytes express a range of neurotransmitter receptors and voltage-gated channels which initiated the idea that astrocytes have a different function apart from tethering the neurons and helping neuronal distribution and interactions. However, previously unrecognized and surprising functions of astrocytes have been investigated only recently, showing that astrocytes actively control CNS homeostasis and ionic buffering, form tripartite synapse with neurons, help in adult neurogenesis, and alter the brain vascular tone. Astrocytes are reported to modulate synaptic transmission by a plastic astrocyte-neuron partnership, which is directly evidenced from imaging studies of the brain slices. Recent studies show that astrocytes electrically not only communicate with ion channels but also maintain ionic and nutrient homeostasis with the help of gap junction (GJ) proteins. Astrocytes are majorly involved in the propagation of ICWs and K+ shunting and maintain blood-brain barrier (BBB) permselectivity with the help of GJs and soluble mediators. Astrocytic end-feet connect the blood capillary endothelial cells (BCECs) and associated basement membrane in BBB to neurons and other CNS resident glia. Astrocytic end-feet released soluble factors like IL-6, interferon IFN-β, TNF-α, IL-1β, and GJ activity directly control BCEC-expressed tight junction (TJ) proteins, which are the structural units of the BBB. Thus, astrocytes communicate between CNS parenchyma and BBB, maintain the integrity of BBB, and control homeostasis during neural network excitability (Khakh and Sofroniew 2015). The localization of astrocytic and oligodendrocytic GJ channels and their coupling partners are shown in C-4 Table 1 and represented as a diagram in Fig. 1 (Table 1).

The neuronal action potential firing increases K+ concentration in the extracellular space. Following this, excessive K+ ions diffuse with the help of oligodendrocytic inward rectifier K+ channels (Kir4.1) and Na+/K+ pump, and this is associated with passive uptake of water through aquaporin 4 channels. These Kir channels help in siphoning excessive K+ ions released during neuronal activity, which is "anomalous" in comparison to the well-known outwardly rectifying K+ current (Lu 2004; Neusch et al. 2001). The strongly negative resting potential and relatively high permeability to K+ of the astrocytes help in the panglial diffusion of K+ ions from extracellular space, where the concentration of K+ is excessive after neuronal activity (Menichella et al. 2003). This way, K+ is redistributed in the oligodendrocyte to astrocytic networks with the help of GJs. The GJ proteins, connexins (Cxs), play a crucial role in this aspect. Oligodendrocytic paranodal Cxs and Cx32/Cx32 channels present in compact myelin carry the excess K+ to oligodendrocyte cell body, and finally it enters in astrocytes majorly via Cx43/Cx47 channels or via few of Cx30/Cx32 channels and spreads away in panglial

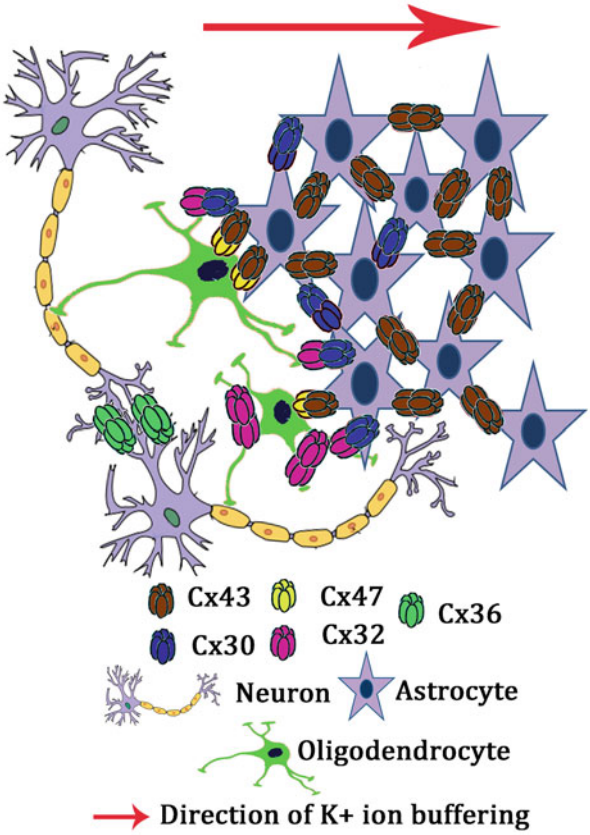

Fig. 1 GJ communication in panglial network. Astrocytes mainly express Cx43, Cx30, and Cx26, but the expression of Cx26 has been debated. Cx43/Cx43 and Cx30/Cx30 form homotypic channels between astrocytes. Oligodendrocytes express Cx47, Cx32, and Cx29. Cx47/Cx43 and Cx32/Cx30 form heterotypic GJCs between oligodendrocytes and astrocytes in both oligodendrocytic somata and proximal processes. Astrocytic/oligodendrocytic Cx30/Cx32 channels are mainly localized along myelinated fibers, whereas the Cx43/Cx47 channels are mainly observed around oligodendrocyte somata. Cx32/Cx32 GJCs are seen along large myelinated fibers in white matter. Cx29 forms homotypic channels mainly at the juxtaparanodes and adaxonal fibers (not shown). Neurons mainly express Cx36, which forms homotypic channels between neurons. Cx43/Cx47 and Cx30/Cx32 channels are important in dispersion of K+ ions from oligodendrocytes to astrocytes. The direction of K+ ionic buffering has been demonstrated by arrow

network. These GJs also play a crucial role in supplying lactate to oligodendrocytes and neurons, during deprivation of energy. Astrocytes can store glycogen, as reserved energy source in CNS. The astrocytes have been shown to express small molecule growth factors like neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF), which help in neuronal survival (Volterra and Meldolesi 2005). Recent in vitro studies demonstrated astrocytes promote oligodendrocytic MOG (a constituent of myelin) formation and the survival of oligodendrocytic precursor

cells (OPCs) by leukemia inhibitory factor (LIF)- and platelet-derived growth factor (PDGF)-dependent signaling (Kiray et al. 2016). Other astrocyte-derived molecules like ciliary neurotrophic factor (CNTF), NT-3, and insulin-like growth factor-1 (IGF-I) also help in OPC survival and maturation (Nair et al. 2008).

Astrocytes also mediated the reuptake of glutamate (another major excitatory neurotransmitter in CNS), which is further dispersed through the panglial network. Quenching of the excess glutamate prevents excitotoxic injury of the neurons. Inside the astrocytes, glutamate is metabolically converted to glutamine, which is further shuttled back to presynaptic nerve terminals. Thus, the astrocytes not only remove excessive neurotransmitters and ions from the extracellular space but also provide metabolic support and supply molecular substrates important for neurotransmission. This way, neurons are protected from excitatory damage, large depolarizations, and metabolic deprivation, absence of which would critically damage the neuronal survival (De Bock et al. 2013).

Distinct Ca2+ signals regulate different pathways like alter blood vessel diameter; control the release of synaptogenic and trophic factors; regulate gene expression, further modulating K+ and neurotransmitter uptake; and control neuronal synchronization. Astrocytes promote neurovascular alterations via vasomodulators like prostaglandins (vasodilation/vasoconstriction) and by sending rapid coordination signals. Neuronal activity-dependent astrocytic intercellular calcium waves (ICWs) were reported to induce vasodilation mediated by cyclooxygenase eicosanoids like prostaglandin E or 20-hydroxyeicosatetraenoic acid release. Astrocytes are now referred to as "excitable" cells because internal or external signals can activate the astrocytes and result in "gliotransmission," inducing a signaling cascade in the neighboring cells. The neuronal signaling and "spillover" of various transmitters and signaling mediators (e.g., acetylcholine, noradrenaline, glutamate, GABA, dopamine, ATP, nitric oxide, and BDNF) induce neuron-dependent activity of astrocytes, including neurotransmitter quenching, processing, and maintenance of ionic and water equilibrium. The quenching of neurotransmitters induces upregulation of intracellular Ca2+ in astrocytes. Gliotransmission also releases different stimulators like neurotransmitters (glutamate, GABA, ATP, dopamine, noradrenaline), TNF-α, and prostaglandin which work on neurons and other glial cells in a stimulus-dependent manner. Astrocytes also control inhibitory synaptic transmission (potentiation of the GABA-containing interneurons in the hippocampal stratum radiatum and CA1 area), which takes place in response to repetitive firing of interneurons. Astrocytes also mediate the stimulation of postsynaptic neuronal excitability. The neurotransmitter like D-serine released by astrocytes acts on hippocampal CA1 pyramidal cell synapses, which, in turn, modulate NMDAR-dependent long-term potentiation (Volterra and Meldolesi 2005). Astrocytes perform these functions with the help of different modes of action like exocytosis, volume-regulated anion channels, purinergic P2X receptors, and GJs.

In summary, astrocytes perform various cell biological functions like maintain ionic (K+, Ca2+) homeostasis and release energy substrates (lactate), transmitter precursors or transmitters, and growth factors. They also uptake several neurotransmitters at the tripartite synapse. To provide nutrient support and ionic

homeostatic control, astrocytes uptake water and glucose from BBB and secrete vaso-active compounds like prostaglandin, arachidonic acid, and NO, which controls the cerebral blood flow.

4 Glial Biology in Neurodegeneration and Demyelination

Gliotransmission and astrocyte-dependent signaling defects are affected in different pathological conditions. For example, Ammon's horn sclerosis is a type of epilepsy that manifests neuronal death and reactive gliosis in hippocampal Ammon's horn area. In patients with Ammon's horn sclerosis, glutamatergic receptor-dependent astrocytic hyperexcitability is observed. The activation of microglia during pathological condition is also associated with the clustering of astrocytes, and the resulting inter-glial interaction leads to massive TNF-α release. This phenomenon, in association with immune response, also amplifies astrocyte-mediated glutamate release and exerts neurotoxic effect (Seifert et al. 2004). Healthy adult astrocytes protect amyloid-β (Aβ)-mediated plaque formation. In Alzheimer's disease, abnormal expression of the enzyme β-secretase by astrocytes and a defect in astrocytic Aβ-degrading properties promote astrocyte-mediated Aβ accumulation. Apolipoprotein E-dependent signaling is predicted to be crucial for preventing accumulation of Aβ. In addition, reactive astrocytes near β-amyloid plaques exerted purinergic receptor-mediated enhanced Ca2+ signaling (Koistinaho et al. 2004). In a subset of familial cases of a fatal upper and lower motor neuron disease, amyotrophic lateral sclerosis (ALS) is associated with point mutation of Cu/Zn superoxide dismutase (SOD1). Interestingly, only if the enzyme is mutated in both neurons and astrocytes, the motor neuron pathology appears, but point mutation only in neurons does not cause pathological changes (Clement et al. 2003). HD patients show nuclear inclusions of mutant huntingtin protein in striatal astrocytes, and significant reductions in astrocytic functional proteins like glutamate transporter (GLT-1 or EAAT2) and potassium channel (Kir4.1) are observed. Gliomas, characterized by malignant transformation of glial cells (mainly astrocytes), induce destruction of surrounding tissues to gain space needed for the expansion of the tumor. Altered astrocytic pathology releases excess glutamate, which is combined with depleted glutamate reuptake causing neurotoxicity in this condition (Sontheimer 2003). During the course of ischemia, trauma, or inflammation, astrocytes exhibit transcriptional changes which has prominent gradient, which varies with distance from lesions and with intensity of tissue injury (Giaume et al. 2010).

In different pathological conditions showing dysmyelination, leukoencephalopathy, and demyelination, astrocyte morphology and functional properties alter. In human CNS demyelinating disease, MS, alteration of astrocytic morphology is a major pathological hallmark, which is termed as "astrogliosis" or "glial-scar formation" (Kuhlmann et al. 2008; Sofroniew and Vinters 2010). Around the demyelinated MS plaques, astrocytes form highly filamentous processes with increased expression of astrocytic markers (e.g., GFAP and vimentin), which is called glial scar.

The glial scar formation is beneficial for restricting the spread of tissue damage during neuroinflammation, in restricting dispersion of toxic substances and apoptotic signals in localized areas. In contrast, astrogliosis significantly alters metabolic coupling in panglial network and prevents OPCs to enter and remyelinate. The alteration of metabolic coupling in panglial network during neuroinflammation and demyelination is the prime focus of this study.

5 The Biology of Gap Junctions

Cell-to-cell communication portrays a pivotal role in the survival of multicellular organisms. Cells communicate directly or anchor to neighboring cells or to extracellular matrix with the help of cell junctions. In vertebrates, mainly three types of cell junctions are present, namely, adherens (or anchoring) junctions, tight junctions, and gap junctions (GJs). The gap junction channels (GJCs) are important in all vertebrates for maintaining cellular homeostasis as GJCs play a pivotal role in the direct diffusion of ions, nutrients, and small molecules (<1kD), including inositol 1,4,5-triphosphate (IP3), cyclic nucleotides, and ATP between cells (Charles et al. 1991, 1992; Finkbeiner 1992; Saez et al. 1989).

Astrocytes express different water channels, ion channels, gap junctions (GJs), and neurotransmitter receptors, which help the astrocytes to maintain metabolic coupling that includes tissue homeostasis, ionic shuttling, and osmotic balance. GJs are most important because they not only mediate direct diffusion of ions and other small molecules but they also interact with a myriad of signaling proteins which control cellular signaling in different conditions. GJs maintain a huge variation of expression, function, and signal transduction, depending on intra- or extracellular stimuli.

GJs are made up of connexin (Cx) proteins. Each Cx protein has four transmembrane domains, two extracellular loops, one cytoplasmic loop, one N-terminal tail, and one C-terminal domain. Six Cx proteins form a hexamer or connexon, which is docked onto the cell membrane of a single cell and forms a hemichannel (HC). Upon docking of another HC from an apposing cell, a transmembrane channel forms, which is called gap junction channels (GJCs). GJCs assembled into higher-order structures in the plasma membrane, which is called gap junction plaque (GJP). In Fig. 2, we show a schematic diagram of Cx proteins and how they form GJICs on cell membrane. The different types GJICs based on oligomerization (homomeric and heteromeric) and opposing channel formation from neighboring cells (homotypic and heterotypic) are shown there.

More than 20 different Cx types have been discovered to be present in humans. All the Cxs are named based on their molecular weight, which is predicted from their cDNA sequence. For example, the most widespread connexin species has a predicted as well as biochemically determined molecular mass of 43 kDa and therefore is designated Cx43. Another alternative nomenclature exists for the Cxs, which is based upon genetic similarity and order of discovery (alpha, beta, gamma, delta, and epsilon groups). The common 20 human Cxs are divided into two major

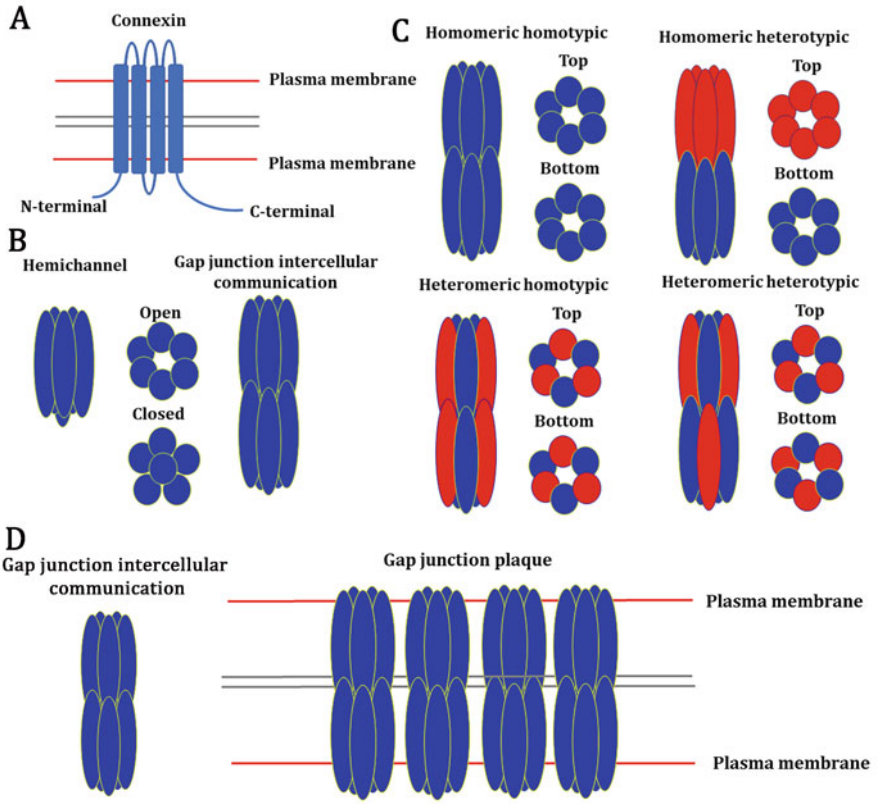

Fig. 2 Different combinations of GJICs comprised of Cx proteins inside the cells. Cx proteins have four transmembrane, two extracellular loops, and one N- and one C-terminal domain (Panel A). Six Cx proteins form a GJ hemichannel on cell surface that can be open or closed depending on the physiological state of the cell/cellular signaling. Two GJ hemichannels from opposing cells can form a complete channel consisting 12 Cx monomers and are named as GJC or GJIC (Panel B). The combination of 12 Cx proteins can vary depending on the type of oligomerization inside the same cell (homomeric or heteromeric) and from juxtaposed cell (homotypic or heterotypic). The front, top, and bottom views of these types of GJICs are represented in Panel C. Several GJICs, localized in closed proximity at the cell surface, form GJ plaques (Panel D)

subgroups, α and β (according to their sequence aligned using ClustalW and eliminating terminal domains), with an additional group of connexins having intermediate sequence homology. According to this nomenclature, Cx43, which is an α-connexin, is alternatively named as GJA1. As individual GJICs are formed by 12 Cxs, multiple types of Cxs can produce mixed channels, given that the Cxs be compatible for hetero-oligomerization. Hence, depending on the composition of Cxs, GJs can be homomeric (composed of the same Cx isoforms, inside the same cell) or heteromeric (composed of different Cx isoforms, inside the same cell) and homotypic (composed of the same Cx protein from two apposing cells) or

heterotypic (composed of different Cx protein from two apposing cells). This heterogeneity of GJ formation by Cxs confers the variation of GJ function between cell and cell, which has difference in signaling, gating properties, and permeability of small molecules (Koval 2006).

Beyond GJICs, Cxs can also form unapposed hemichannels (HCs), which can be present both as GJ precursors at the cell surface and as permanent non-junction channels, which are never incorporated into GJs. Previously, it was thought that HCs are unstable intermediate form at the cell surface and remain closed until they form a GJIC because uncontrolled HC opening leads to leakage of essential small molecules and substantial membrane depolarization, leading to cell dysfunction/death. Recent studies show that HC opening is mediated by different molecular stimuli and HCs itself are involved in different types of paracrine signaling (mediated by the release of ATP, glutamate, glutathione, NAD+, and prostaglandins) (Wang et al. 2013; Willebrords et al. 2016). HC opening is controlled by various cell signaling molecules, interacting with the C-terminal domain of Cx. Astrocytic HC opening/activation may lead to gliotransmitter release, which, in turn, propagates excitatory as well as inhibitory signaling (De Bock et al. 2013). The importance of HCs is currently being investigated thoroughly. GJs perform similar functions of small molecule diffusion as well as participate in cellular signaling through their C-terminal tail and mediate communication between two neighboring cells. The direct diffusion of small molecules along GJs and HCs helps in the coordination of synergistic cellular function in several types of organs and tissues. GJCs help in the propagation of ICWs, metabolic and electric coupling in CNS glial cells and cardiomyocytes, exchange of bone-modulating molecules, and synchronization of the smooth muscle cell contraction (De Bock et al. 2013).

6 Properties of Gap Junctions: Synthesis, Oligomerization, Trafficking, and Degradation

In different genetic as well as sporadic diseases, the synthesis, oligomerization, trafficking, post-translational modification, or degradation of GJs is observed to be altered. The mutation of CNS GJ proteins giving rise to dysmyelinating conditions (discussed in detail in the next section) has functional alteration in one or more than one such processes. Thus, these processes bear a pivotal importance in the normal functioning of GJs.

Cxs are synthesized at the endoplasmic reticulum (ER) membrane in a process involving signal recognition peptide (SRP) and internal signal sequence interaction, docking of peptide/ribosome complex to translocon, and cotranslational integration of Cx peptides in ER membrane. Functional transmembrane topology is obtained during ER membrane integration. Previous studies show Cxs oligomerize in ER membrane or ER Golgi intermediate complex (ERGIC) for β-Cxs like Cx32. In contrast, α-Cxs like Cx43 are reported to be oligomerized later in the late Golgi membrane (Kumar and Gilula 1996) or upon exit from the trans-Golgi network (Das Sarma et al. 2001). Assembly and oligomerization is an obvious prerequisite for

further Cx trafficking to the cell membrane. Upon cell membrane delivery, Cxs are terminally phosphorylated and form clusters of GJPs.

The oligomerization process of Cx is believed to be more complex, as different Cx isotypes do not assemble in random order, but interact selectively aiding homomeric or heteromeric oligomerization of Cxs. For example, α-Cxs can only form heteromeric Cx with another α-Cxs but not with any other Cxs belonging to other subgroup. Experimentally it is seen that cotransfection with Cx32 (GJβ1) and Cx26 (GJβ2) gives rise to heteromeric plaques colocalized at the cell surface, but Cx43 (GJα1) and Cx26 (GJβ2) cannot make such plaques (Koval 2006). Thus, all heteromeric channels are formed by two different Cx types, but these Cxs must belong to same subgroup. To date, there is no report that Cxs from different subgroup can form a heteromeric GJC and this phenomenon is named "innate heteromeric incompatibility." The formation of heterotypic GJCs by a head-to-head interaction of Cx hemichannels is not always limited by the different α or β subgroups to which the Cxs belong to. For example, Cx43 (GJα1) is not compatible to form heterotypic GJCs with Cx32 (GJβ1), but Cx46 (GJα3) can form heterotypic channels with both Cx43 (GJα1) and Cx32 (GJβ1). Few of the Cxs, like Cx36, are known to only form homotypic channels. However, inter-group heteromeric channels are less common (Bai and Wang 2014; Koval et al. 2014). The synthesis and folding of Cx43 proteins inside ER, oligomerization in Golgi bodies, and MT-dependent delivery are represented in Fig. 3, which also depicts the formation of Cx43/Cx43 or Cx43/Cx47 channels between juxtaposed cells. Figure 4 shows the typical Cx43 puncta in the cell surface of GFAP+ astrocytes and perikaryonic stain of Cx47 in oligodendrocytes in the mouse brain.

Cx proteins may be post-translationally modified at different sites at C-terminal, before and after delivery to the cell surface, of which the most important is phosphorylation. Phosphorylation takes place inside the vesicular carriers as well as at the cell surface and creates docking sites for different cell signaling molecules. Phosphorylation events of Cxs are also essential for the proper control of the formation and modulation of functional GJCs. It has been observed that at least nine types of Cxs (Cx31, Cx32, Cx37, Cx40, Cx43, Cx45, Cx46, Cx50, and Cx56) are essentially phosphorylated, while, in contrast, others, such as Cx26, remain non-phosphorylated. As discussed, Cx phosphorylation is mediated by different kinases such as Src, protein kinase C (PKC), and mitogen-activated protein kinases (MAPKs). Polyacrylamide gel electrophoresis (SDS-PAGE) demonstrates that Cx43 has different phosphorylated isoforms, including a faster migrating non-phosphorylated form of Cx43 (P_0 or NP; 42 kDa), and primarily two slower migrating isotypes, commonly termed P1 (approximately 44 kDa) and P2 (approximately 46 kDa) forms of Cx43. Upon treatment with alkaline phosphatase, both P1 and P2 isoforms co-migrate with P_0 isoform, suggesting that these Cx isoforms of Cxs primarily arise from phosphorylation. Most of these covalent modifications, as indicated by phosphoamino acid analysis, are shown to occur on serines, although tyrosine phosphorylation of Cx43 is reported to be mediated by activated pp60src. A fraction of monomeric Cx43, which is mainly found before TGN, is phosphorylated and migrated at the P1 position. This phenomenon proves that transient

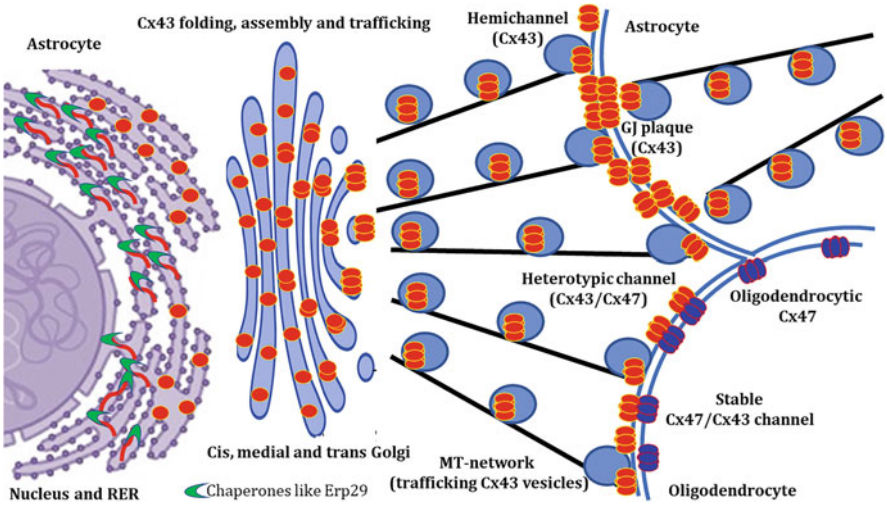

Fig. 3 Synthesis, oligomerization, and trafficking of Cx43 proteins that form Cx43/Cx43 or Cx43/Cx47 channels on cell surface. In this schematic image, we represent the synthesis of Cx43 proteins inside the ER (red, curved lines) and folded (red, ellipse) inside ER/ERGIC with the help of chaperones (green). In the Golgi bodies, Cx43 proteins are oligomerized and then hexamerized (in the TGN: red, hexamers) that is finally carried along the MTs (black lines) with the help of vesicles (blue). Finally, the Cx43 containing vesicles fuse onto cell membrane to deliver GJs on cell surface where they can form Cx43/Cx43 homomeric homotypic channels with neighboring astrocytes or Cx43/Cx47 homomeric heterotypic channels with neighboring oligodendrocytes

phosphorylation may take place prior to Cx43 delivery to the cell surface; however, it is not a prerequisite for Cx43 intracellular trafficking and delivery to the cell surface. In addition, some non-phosphorylated moiety of Cx43 can also be found at the cell membrane. The terminal phosphorylation of Cxs to their final phosphorylated form (P2) is associated with their assembly in GJPs and confers resistance to detergents like Triton X-100 solubilization. Taken together, HC and GJC phosphorylation events are affected by several kinase pathways, which determine the stability, the degradation, as well as the gating and signaling mediated by Cxs (Solan and Lampe 2005).

It is important that delivery and Cx stability in GJPs depend on its interacting partners. Current studies elucidated the role of microtubules (MTs) on Cx43 delivery to the cell surface. Time-lapse imaging revealed that Cx43 is delivered in vesicular carriers traveling along MTs, which mediate the delivery of Cx43 from the Golgi network to the plasma membrane. Subcellular fractionation studies and immunolabeling followed by colocalization analyses demonstrated that Cxs traffic through the Golgi compartment and the vesicular transport along microtubules (MTs) deliver Cxs to cell surface. Upon reaching cell surface, Slatternly move, dock, and fuse at the outer plaque regions to form a complete GJP. It is seen that a number of small vesicles, containing Cxs, traffic toward the + end of MTs, which are finally extended and fuse onto non-junctional plasma membrane in the cell periphery

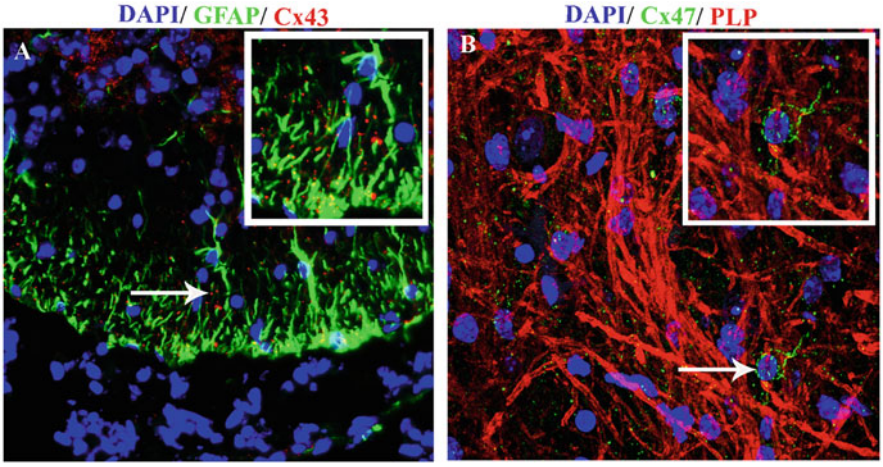

Fig. 4 Astrocytic expression of Cx43 and oligodendrocytic expression of Cx47 in healthy mouse CNS. In normal mouse brain, Cx43 punctate staining is observed around glial fibrillary acidic protein (GFAP; astrocyte marker)+ astrocytes (arrow, Panel A, Cx43, red; GFAP, green), whereas the PLP+ myelinated nerve fibers are surrounded by oligodendrocyte cell bodies having perikaryonic (string-on-beads appearance) appearance of Cx47 (arrow, Panel B, Cx47, green; PLP, red). Counterstain of DAPI shows the nuclei in both the images (blue). Insets demonstrate more detailed visualization of cell-specific localization of Cxs

(Lauf et al. 2002). Similar MT-mediated Cx43 trafficking was observed in mouse embryonic fibroblasts (MEFs). As GJs tether MT elements at the surface, deficiency of Cx43 also induces loss of cellular directional movement and cell polarity, which is associated with the disorganization of MT organization center (MTOC) and Golgi apparatus. This study suggests the Cx43 β-tubulin binding domain is necessary for normal directional arrangement and regulation of the MT network (Francis et al. 2011). A very thorough study performed by Shaw et al. (Shaw et al. 2007) investigated the mechanism of Cx43 delivery to the cell surface. Among the MT plus-end-tracking proteins (+TIPs), EB1 is identified as a major player in GJ formation. Dimeric EB1 has dual binding sites for many proteins, including p150 (glued), which is a component of the dynein/dynactin complex. The dynein/dynactin complex, in turn, tethers MTs to N-cadherin-mediated adherens junctions (AJs). In this manner, cortical capture of MTs by AJs allows the localized deposition of Cx43 HCs into the plasma membrane. In brief, Cx43 containing vesicular carriers are associated with EB1, p150 (glued) of dynein/dynactin complex, and β-catenin, which links MTs to the cell surface and mediates the focal delivery of Cx43 HCs to the plasma membrane (Shaw et al. 2007). Catenins and cadherins are also important for the stability of Cxs in GJ plaques. Cx43 directly interacts with MTs at the cell surface. A 35-amino acid containing juxta membrane region in the Cx43 C-terminal tail with a tubulin binding motif is reported to be necessary and sufficient for MT-binding (Giepmans et al. 2001). Cx43 anchors MT growing (+) ends to GJs with the help of 234KGVKDRVKGK243 sequence (in rats) on the Cx43 tail.

7 Functional Importance of Astrocytic and Oligodendrocytic Gap Junctions

Several cell biological studies help to elucidate the role of astrocyte/oligodendrocytic GJ communication mediated by Cx43/Cx47 channels. These studies elaborate the role of Cx43/Cx47 channels for maintaining ionic and nutrient buffering inside the CNS, which, in turn, controls oligodendrocyte morphology and function. It was found that astrocytes slowly depolarize when the amphibian optic nerves are stimulated by the upregulation of extracellular K+ concentration. Thus, Orkland et al. (1966) proposed that GJs aid in the spatial buffering of K+ ions, which are released during neural activity. This finding specifically indicated the involvement of neurons to the previously proposed "spatial buffer theory," proposing the involvement of astrocytes only in a highly coupled network, but the involvement of other glial cells or neurons was not considered. It was also found that GJs facilitate siphoning of K+ in retinal astrocytes during neuronal activity, further helping in the propagation of inter-astrocytic Ca2+ waves. This is important in glial-neuronal signaling, by providing a pathway for lateral diffusion and cell-to-cell dispersion of these ions (Zahs and Newman 1997). However, current evidence suggests that oligodendrocytes are also connected to this astrocytic network, which might be helpful to improve the K+ spatial buffering.

Astrocytes are permeable to K+ ions and are coupled to the neighboring astrocytes, which enables the redistribution of extracellular K+. A part of the potential mechanisms for K+ redistribution has been studied in the brain of mice (Kofuji and Newman 2004), but the role of GJs was not elucidated. Later, a study by Wallraff et al. (2006) showed the deletion of astrocytic Cx43 and Cx30 affected K+ buffering in the brain gray matter. These experiments demonstrated the role of astrocytic GJs in accelerated K+ clearance, limiting K+ accumulation during synchronized neuronal firing, but a mechanistic difference in gray and white matter was not elaborated. Thus, K+ ions disbursed from myelinated axons are likely to be accumulated in the periaxonal space, followed by its dispersion by entering axons and enwrapping oligodendrocytic processes via Na + K + ATPases (Ransom et al. 2000) or possibly by diffusion through paranodal GJs. Once K+ enters the somatic region of an oligodendrocyte, it may laterally diffuse via reflexive Cx32/Cx32 GJCs and then enter astrocytes via astrocyte/oligodendrocyte channels mediated by Cx32/Cx30 and Cx43/Cx47 GJ proteins (Orthmann-Murphy et al. 2007). Cx47-positive puncta are aligned along proximal processes of oligodendrocytes in a "beads-on-a-string" manner suggesting Cx47 is associated with myelinating fibers. Astrocyte/oligodendrocyte GJCs composed of Cx43/Cx47 are primarily localized in the oligodendrocyte somata and proximal processes, whereas astrocyte/oligodendrocyte Cx30/Cx32 GJCs are mainly concentrated in the myelin lamellae and fewer in the oligodendrocyte somata. Cx32/Cx30 and Cx47/Cx43 channels have distinct and voltage-gating properties and small molecular dye permeability. Compared to the Cx32/Cx30 channels, Cx47/Cx43 GJCs are more symmetrical in their permeability properties. In the oligodendrocytic somatic regions, mainly Cx47/Cx43 are found, and Cx32/Cx30 fewer channels are observed, which are primarily localized in the

gray matter. Thus, they might be involved in a fast dispersal of K+ ions from oligodendrocytes to astrocytic network in white matter. Hence, depletion of Cx47-mediated channels cannot be replenished completely by Cx30/Cx32 channels, and Cx32/Cx30 channels take part only in directional oligodendrocyte/astrocyte K+ buffering, restricted to gray matters.

Thus, Cx43/Cx47 channels are crucial for the redistribution of K+ from oligodendrocytic somata to neighboring astrocytes. Upon loss of Cx43/Cx47 channels, distribution of glutamate and K+ is altered in the oligodendrocyte/astrocyte networks. This phenomenon leads to a local accumulation of toxic substances and water, inducing swelling of astrocytes (edema), decreased extracellular space volume, increase in K+ and glutamate concentration, and accumulation of neurotoxic substances, which is sensed by neurons. Reduced expression of Cx43 is observed to increase glutamate transporter expression (Unger et al. 2012), but the feedback mechanism and functional importance are not clear. However, upon silencing of the Cx43 and Cx30 expression in the astrocytes, the clearance and redistribution of K+ ion are partially maintained in the hippocampal area (Wallraff et al. 2006). So other GJ-independent mechanisms might be operative, which contribute to spatial buffering. In addition to altered ionic and small molecule concentration, the disruption of CNS Cxs also induces BBB leakage and entry of other circulating compounds in the brain parenchyma and causes the upregulation of GFAP which is reflected in astrogliosis, swelling of the cells, and abnormal proliferation (David et al. 2009; De Bock et al. 2013).

Cx43/Cx47 channels are also shown to be important for nutrient homeostasis. Rouach et al. (2008) showed that astrocytic GJs in the CNS mediate intercellular trafficking of blood-derived glucose and its metabolites, which portrays an important functional role in the releasing of lactate to the neurons. Thus, in the absence of GJ coupling, entrapped intracellular carbohydrate and its metabolites might create an osmotic gradient, and water could also follow the gradient to induce cellular edema (Lutz et al. 2009). When extracellular glucose is depleted, astrocytic GJs mediate the delivery of glucose or lactate to neighboring glial cells upon glutamatergic transmission. The glutamate, which is released due to spontaneous, evoked, or pathological epileptiform activity, enhances the trafficking of glucose into the astroglial/panglial networks. Also, lactate can diffuse through astrocytic Cx43- and Cx30-mediated GJs, which can be used by neurons for providing energy to sustain their excitatory synaptic transmission. Thus, the GJs directly help in astrocyte-mediated energetic support to the distal neurons, which cannot receive glucose or its metabolites directly from the blood vessels (Rouach et al. 2008). Cx43/Cx47 channels are also hypothesized to be important for the delivery of such nutrients to oligodendrocytes (Morrison et al. 2013). Currently, the molecular basis and importance of the formation of GJIC-mediated panglial syncytium are a prime choice of investigation among the cell biologists.

Not only through Cx43/Cx47 channels, astrocytic GJs may also be involved in other signal transduction pathways which have a crucial role in the maintenance of oligodendrocyte health and retention of normal myelin. ATP is liberated from axons that are firing action potentials. This ATP enters the astrocytes. Astrocytes, in turn,

produce a cytokine leukemia inhibitory factor (LIF). LIF acts on the oligodendrocytes, helping in the production of myelin oligodendrocyte glycoprotein (MOG), and promotes myelination (Ishibashi et al. 2006). Astrocytic HCs as well as GJICs play a crucial role in the propagation of ATP in normal or physiological conditions (Willebrords et al. 2016). Hence, astrocytic communications beyond Cx43/Cx47 channels may be crucial in the maintenance of myelin, through different soluble mediators.

8 Conclusion

8.1 Gap Junctions as a Novel Target in Demyelinating Diseases

Recent efforts investigating the role of different CNS-specific GJ knockouts and alteration of GJs associated with neuroinflammation and demyelination suggest that the loss of Cx43/Cx47 functional channels seems to be crucial for the maintenance of myelin and loss of Cx43/Cx47 channels provides a mechanism by which chronic demyelinating plaques expand during SP-MS. All these studies provide enough evidence that remodeling of panglial GJ network not only is closely associated with pathological alterations seen in and around chronic MS lesions but also is present in the normal-appearing white matter (NAWM). The altered glial connectivity in NAWM is likely to cause environmental alterations, which might directly help in axonal loss, demyelination, and spreading of MS lesions. Whether Cx32 and Cx30 are also crucial and mechanistically important can be a prime choice for further investigation. Whether restoration of the GJ coupling between astrocytes, astrocyte/oligodendrocyte, and OPCs can be used to stop the progression of MS can be debated. Recently, Cxs are seen as a therapeutic target in different diseases. For example, malignant astrocytic gliomas or glioblastoma (astrocytoma grade IV), which is among the most lethal intracranial tumors, shows loss of Cx43. The HSVtk results in bystander cell death of tumor cells in a Cx43-dependent manner upon treatment with ganciclovir and has been implicated in glioblastoma treatment (Asklund et al. 2003; Cirenei et al. 1998; Huang et al. 1999). Cx43 targeted with α-Cx carboxyl-terminal peptide in a breast cancer model showed enhanced activity of breast cancer-specific drugs like tamoxifen and lapatinib (Grek et al. 2015). In contrast, inhibition/uncoupling of Cx43-mediated channels has been targeted as an alternative approach to the drug-induced inhibition of cardiac sodium channels in cardiac diseases (Burnham et al. 2014).

A number of dephosphorylating agents, phorbol esters, PKC inhibitors, and eicosanoids, are already established as GJ uncoupling agents, but lack of Cx-specific activity and more generalized effects exerted by these molecules limit their application as targeted therapeutic agents. On the other hand, cAMP, forskolin, and isoprenaline, antiarrhythmic drugs like tedisamil, eicosanoids like 11,12-epoxyeicosatrienoic acid, phorbol ester like TPA, and 5-hydroxytryptamine histamine are reported to enhance GJ activity (Salameh and Dhein 2005). Although most of the studies are done on Cx43, these agents also lack the specificity on targeting a

specific Cx. In addition, till date, no study has been performed on demyelinating diseases specifically MS.

The demyelinating diseases show restriction of Cxs to the cell surface, and this is seen in a viral model of MS. We are the first to report the mechanism of altered Cx43 trafficking and its retention in ER/ERGIC. It may be important to improve and enhance Cx43 delivery to cell surface. ERp29 is reported to be an important chaperone, which helps in the oligomerization and folding of Cx43 in the intracellular compartment and enhances Cx43 delivery to the cell surface (Das et al. 2009). Cx43 also interacts with other chaperones like HSP-70 (Hatakeyama et al. 2013). Sodium salt of an aromatic fatty acid, 4-phenylbutyrate (4-PBA), is reported to increase the level of GJ-associated chaperones (Molina et al. 2015; Suaud et al. 2011). Hence, upregulation of GJ delivery to the cell surface may be a novel therapeutic approach (currently being investigated in our laboratory), but tissue- and cell-specific delivery and targeting a specific Cx remain as potent challenges.

Acknowledgments Supported in part by research grants (BT/PR14260/MED/30/437/2010 and BT/PR4530/MED/30/715/2012) from the Department of Biotechnology, India; research grant (RG3774A2/1) from the National Multiple Sclerosis Society (NMSS), USA; and the Indian Institute of Science Education and Research-Kolkata (IISER-K), India, start-up fund to JDS. The Council for Scientific and Industrial Research (CSIR), India, and Du Pré Grant from the Multiple Sclerosis International Federation (MSIF) provided the research support to RB.

Competing Interests The authors declare that they have no competing interests.

References

Asklund T, Appelskog IB, Ammerpohl O, Langmoen IA, Dilber MS, Aints A, Ekstrom TJ, Almqvist PM (2003) Gap junction-mediated bystander effect in primary cultures of human malignant gliomas with recombinant expression of the HSVtk gene. Exp Cell Res 284:185–195

Bai D, Wang AH (2014) Extracellular domains play different roles in gap junction formation and docking compatibility. Biochem J 458:1–10

Burnham MP, Sharpe PM, Garner C, Hughes R, Pollard CE, Bowes J (2014) Investigation of connexin 43 uncoupling and prolongation of the cardiac QRS complex in preclinical and marketed drugs. Br J Pharmacol 171:4808–4819

Charles AC, Merrill JE, Dirksen ER, Sanderson MJ (1991) Intercellular signaling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. Neuron 6: 983–992

Charles AC, Naus CC, Zhu D, Kidder GM, Dirksen ER, Sanderson MJ (1992) Intercellular calcium signaling via gap junctions in glioma cells. J Cell Biol 118:195–201

Cirenei N, Colombo BM, Mesnil M, Benedetti S, Yamasaki H, Finocchiaro G (1998) In vitro and in vivo effects of retrovirus-mediated transfer of the connexin 43 gene in malignant gliomas: consequences for HSVtk/GCV anticancer gene therapy. Gene Ther 5:1221–1226

Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillee S, Rule M, McMahon AP, Doucette W, Siwek D, Ferrante RJ et al (2003) Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science 302:113–117

Das S, Smith TD, Sarma JD, Ritzenthaler JD, Maza J, Kaplan BE, Cunningham LA, Suaud L, Hubbard MJ, Rubenstein RC et al (2009) ERp29 restricts Connexin43 oligomerization in the endoplasmic reticulum. Mol Biol Cell 20:2593–2604

Das Sarma J, Meyer RA, Wang F, Abraham V, Lo CW, Koval M (2001) Multimeric connexin interactions prior to the trans-Golgi network. J Cell Sci 114:4013–4024
David Y, Cacheaux LP, Ivens S, Lapilover E, Heinemann U, Kaufer D, Friedman A (2009) Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis ? J Neurosci 29:10588–10599
De Bock M, Kerrebrouck M, Wang N, Leybaert L (2013) Neurological manifestations of oculodentodigital dysplasia: a Cx43 channelopathy of the central nervous system ? Front Pharmacol 4:10–16
Finkbeiner S (1992) Calcium waves in astrocytes-filling in the gaps. Neuron 8:1101–1108
Francis R, Xu X, Park H, Wei CJ, Chang S, Chatterjee B, Lo C (2011) Connexin43 modulates cell polarity and directional cell migration by regulating microtubule dynamics. PLoS One 6:e26379
Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N (2010) Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 11:87–99
Giepmans BN, Verlaan I, Hengeveld T, Janssen H, Calafat J, Falk MM, Moolenaar WH (2001) Gap junction protein connexin-43 interacts directly with microtubules. Curr Biol 11:1364–1368
Grek CL, Rhett JM, Bruce JS, Abt MA, Ghatnekar GS, Yeh ES (2015) Targeting connexin 43 with alpha-connexin carboxyl-terminal (ACT1) peptide enhances the activity of the targeted inhibitors, tamoxifen and lapatinib, in breast cancer: clinical implication for ACT1. BMC Cancer 15:296
Hatakeyama T, Dai P, Harada Y, Hino H, Tsukahara F, Maru Y, Otsuji E, Takamatsu T (2013) Connexin43 functions as a novel interacting partner of heat shock cognate protein 70. Sci Rep 3: 2719
Huang RP, Hossain MZ, Sehgal A, Boynton AL (1999) Reduced connexin 43 expression in high-grade human brain glioma cells. J Surg Oncol 70:21–24
Ishibashi T, Dakin KA, Stevens B, Lee PR, Kozlov SV, Stewart CL, Fields RD (2006) Astrocytes promote myelination in response to electrical impulses. Neuron 49:823–832
Khakh BS, Sofroniew MV (2015) Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci 18:942–952
Kiray H, Lindsay SL, Hosseinzadeh S, Barnett SC (2016) The multifaceted role of astrocytes in regulating myelination. Exp Neurol 283:541–549
Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. Neuroscience 129:1045–1056
Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J, Higgs R, Liu F, Malkani S, Bales KR et al (2004) Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. Nat Med 10:719–726
Koval M (2006) Pathways and control of connexin oligomerization. Trends Cell Biol 16:159–166
Koval M, Molina SA, Burt JM (2014) Mix and match: investigating heteromeric and heterotypic gap junction channels in model systems and native tissues. FEBS Lett 588:1193–1204
Kuffler SW (1967) Neuroglial cells: physiological properties and a potassium mediated effect of neuronal activity on the glial membrane potential. Proc Royal Soci Lond Ser Biol Sci 168:1–21
Kuhlmann T, Lassmann H, Bruck W (2008) Diagnosis of inflammatory demyelination in biopsy specimens: a practical approach. Acta Neuropathol 115:275–287
Kumar NM, Gilula NB (1996) The gap junction communication channel. Cell 84:381–388
Lauf U, Giepmans BN, Lopez P, Braconnot S, Chen SC, Falk MM (2002) Dynamic trafficking and delivery of connexons to the plasma membrane and accretion to gap junctions in living cells. Proc Natl Acad Sci U S A 99:10446–10451
Lu Z (2004) Mechanism of rectification in inward-rectifier K+ channels. Annu Rev Physiol 66:103–129
Lutz SE, Zhao Y, Gulinello M, Lee SC, Raine CS, Brosnan CF (2009) Deletion of astrocyte connexins 43 and 30 leads to a dysmyelinating phenotype and hippocampal CA1 vacuolation. J Neurosci 29:7743–7752
Menichella DM, Goodenough DA, Sirkowski E, Scherer SS, Paul DL (2003) Connexins are critical for normal myelination in the CNS. J Neurosci Off J Soc Neurosci 23:5963–5973

Molina SA, Stauffer B, Moriarty HK, Kim AH, McCarty NA, Koval M (2015) Junctional abnormalities in human airway epithelial cells expressing F508del CFTR. Am J Physiol Lung Cell Mol Physiol 309:L475–L487

Morrison BM, Lee Y, Rothstein JD (2013) Oligodendroglia: metabolic supporters of axons. Trends Cell Biol 23:644–651

Nair A, Frederick TJ, Miller SD (2008) Astrocytes in multiple sclerosis: a product of their environment. Cell Mol Life Sci 65:2702–2720

Neusch C, Rozengurt N, Jacobs RE, Lester HA, Kofuji P (2001) Kir4.1 potassium channel subunit is crucial for oligodendrocyte development and in vivo myelination. J Neurosci 21:5429–5438

Orkand RK, Nicholls JG, Kuffler SW (1966) Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J Neurophysiol 29:788–806

Orthmann-Murphy JL, Freidin M, Fischer E, Scherer SS, Abrams CK (2007) Two distinct heterotypic channels mediate gap junction coupling between astrocyte and oligodendrocyte connexins. J Neurosci 27:13949–13957

Ransom CB, Ransom BR, Sontheimer H (2000) Activity-dependent extracellular K+ accumulation in rat optic nerve: the role of glial and axonal Na+ pumps. J Physiol 522(Pt 3):427–442

Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C (2008) Astroglial metabolic networks sustain hippocampal synaptic transmission. Science 322:1551–1555

Saez JC, Connor JA, Spray DC, Bennett MV (1989) Hepatocyte gap junctions are permeable to the second messenger, inositol 1,4,5-trisphosphate, and to calcium ions. Proc Natl Acad Sci U S A 86:2708–2712

Salameh A, Dhein S (2005) Pharmacology of gap junctions. New pharmacological targets for treatment of arrhythmia, seizure and cancer? Biochim Biophys Acta 1719:36–58

Seifert G, Huttmann K, Schramm J, Steinhauser C (2004) Enhanced relative expression of glutamate receptor 1 flip AMPA receptor subunits in hippocampal astrocytes of epilepsy patients with Ammon's horn sclerosis. J Neurosci 24:1996–2003

Shaw RM, Fay AJ, Puthenveedu MA, von Zastrow M, Jan YN, Jan LY (2007) Microtubule plus-end-tracking proteins target gap junctions directly from the cell interior to adherens junctions. Cell 128:547–560

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7–35

Solan JL, Lampe PD (2005) Connexin phosphorylation as a regulatory event linked to gap junction channel assembly. Biochim Biophys Acta 1711:154–163

Sontheimer H (2003) Malignant gliomas: perverting glutamate and ion homeostasis for selective advantage. Trends Neurosci 26:543–549

Suaud L, Miller K, Alvey L, Yan W, Robay A, Kebler C, Kreindler JL, Guttentag S, Hubbard MJ, Rubenstein RC (2011) ERp29 regulates DeltaF508 and wild-type cystic fibrosis transmembrane conductance regulator (CFTR) trafficking to the plasma membrane in cystic fibrosis (CF) and non-CF epithelial cells. J Biol Chem 286:21239–21253

Unger T, Bette S, Zhang J, Theis M, Engele J (2012) Connexin-deficiency affects expression levels of glial glutamate transporters within the cerebrum. Neurosci Lett 506:12–16

Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 6:626–640

Wallraff A, Kohling R, Heinemann U, Theis M, Willecke K, Steinhauser C (2006) The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. J Neurosci Off J Soc Neurosci 26:5438–5447

Wang N, De Bock M, Decrock E, Bol M, Gadicherla A, Vinken M, Rogiers V, Bukauskas FF, Bultynck G, Leybaert L (2013) Paracrine signaling through plasma membrane hemichannels. Biochim Biophys Acta 1828:35–50

Willebrords J, Crespo Yanguas S, Maes M, Decrock E, Wang N, Leybaert L, Kwak BR, Green CR, Cogliati B, Vinken M (2016) Connexins and their channels in inflammation. Crit Rev Biochem Mol Biol 51:413–439

Zahs KR, Newman EA (1997) Asymmetric gap junctional coupling between glial cells in the rat retina. Glia 20:10–22

Oligodendroglial Gap Junction Communication in CNS Myelination and Demyelination

Rahul Basu and Jayasri Das Sarma

Abstract

In the previous chapter, we discussed the physiological importance of gap junction proteins, namely connexins (Cxs), and the importance of astrocytes and oligodendrocytes, which form Gap Junction Intercellular Communication (GJICs) to maintain nervous system homeostasis. In this chapter, we will discuss the physiology of glial Gap Junction Intercellular Channels (GJICs) in health and disease. Several genetic and acquired (infectious/autoimmune/unknown etiologic) conditions result in the alteration of Cxs and functionality of GJICs. This chapter discusses the alteration of gap junction protein (GJPs) and its pathological consequences in the altered panglial system. For example, several neurodegenerating, demyelinating, and neuroinflammatory disorders exert the alteration of astrocyte-mediated metabolic coupling.

Similarly, mutations of CNS Cx proteins induce neuroinflammation/demyelination and dysmyelination. The mutation of astrocytic Cx43 and oligodendrocytic Cx47 specifically exerts loss of CNS dysmyelinating diseases. Recent studies elucidate that Cx43/Cx47 metabolic alliance plays a crucial role in myelination. This review mainly focuses on the role of Cxs in dysmyelinating and demyelinating diseases. The loss of Cx43/Cx47 function during neuroinflammatory demyelination and its disease models elucidate the potential of targeting GJs for therapeutic purposes in the future.

R. Basu · J. D. Sarma (✉)
Department of Biological Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur, West Bengal, India
e-mail: dassarmaj@iiserkol.ac.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_5

Keywords

Astrocyte · Oligodendrocyte · Gap junction · Connexin43 · Connexin47 · Metabolic coupling · Dysmyelination · Demyelination · Multiple sclerosis · Mouse hepatitis virus

1 Gap Junctions in the Nervous System

GJs are present in several types of central nervous system (CNS) cells, which are astrocytes, ependymocytes, brain fibroblasts, oligodendrocytes, and neurons. Astrocytes are the primary cell type in CNS, which control CNS homeostasis and ionic and nutrient buffering. GJs are most important in this perspective because they not only mediate direct diffusion of ions and other small molecules but they also interact with a myriad of signaling proteins which help in maintaining nutrient and ionic homeostasis, normal cell biological function, and signal transduction, depending on intra- or extracellular stimuli.

Astrocytes express a profuse amount of GJ proteins, which form GJIC not only between the astrocytes but also between astrocytes and other glial cells. Astrocytes express at least three connexins (Cx43, Cx30, and Cx26; however, the expression of astrocytic Cx26 is debated) (Dermietzel et al. 1989; Kunzelmann et al. 1999; Nagy et al. 2001) and show considerable CNS-specific regional variation in their expression (Nagy et al. 1999). Cx43 is the most prevalent astrocytic connexin both in vivo and in vitro (Naus et al. 1997). The expression of Cx26 in astrocytes is controversial, as deletion of both Cx43 and Cx30 in astrocytes abolished most inter-astrocytic coupling in the mice, causing severe pathological conditions and significant disruption of homeostasis. Thus, Cx26 expression in astrocytes is predicted to be at deficient levels and restricted to some regions of CNS only. In addition, astrocytes expressing Cx26 have also been challenged because reporter gene *lacZ* expression was not detected in the astrocytes under the Cx26 promoter in the murine system (Filippov et al. 2003).

Astrocyte/astrocyte GJ couplings are highly sensitive to different environmental stimuli and physiological conditions. Cx43-mediated channels primarily connect astrocytes. Cx43 is also the most abundantly expressed Cx in the CNS (Giaume and McCarthy 1996; Iacobas et al. 2003). Cx43 is profusely expressed throughout the myelinated white matter regions of the CNS. A high number of Cx43 molecules form homotypic inter-astrocytic GJ. These GJs connect the brain parenchyma to the brain capillaries and the ependymal layer of BBB through astrocytic end-feet, forming a complete network. Another astrocytic GJ protein, Cx30, is mainly observed in gray matter regions. Double-immunolabeling and GJ functional studies show that astrocyte/astrocyte GJs are composed of Cx43/Cx43- and Cx30/Cx30-mediated channels in the gray matter. However, expression of Cx30 in certain white matter areas is observed, representing inter-astrocytic Cx30/Cx30 channels. Cx30-mediated channels are also predicted to participate with oligodendrocytes in this

region by heterotypic channels (Nagy et al. 1999; Rash et al. 2001; Rouach et al. 2002; Rozental et al. 2000).

Oligodendrocytes form GJs mainly with astrocytes and depend on them for the maintenance of homeostasis and nutrient support. Hence, the astrocytes connect to other astrocytes and other brain cells to form a GJ connected network named the "panglial syncytium." Oligodendrocytes mainly express three Cxs, namely Cx29, Cx32, and Cx47. Cx29 usually does not appear in oligodendrocyte somatic regions (cell bodies) and is shown to be incapable of forming functional GJICs with other cells (Nagy et al. 2003). The Cx29/Cx29 channels form small intracellular plaques along with oligodendrocytic processes, particularly myelin sheaths enwrapping smaller axons, in the juxtaparanode (Altevogt et al. 2002). Cx29 HCs are also observed in the internode, along with the small myelinated tracts present in gray and white matter regions. Cx32/Cx32 homotypic channels are observed along the large myelinated fibers of the white matter, in Schmidt-Lanterman incisures, and at paranodes which are at the bordering site of the nodes of Ranvier (forming predominantly intracellular GJCs within the myelin sheath) in CNS, but it does not colocalize with Cx29/Cx29 channels. Cx32/Cx32 channels are also present along the myelin sheath in the peripheral nervous system (PNS).

In contrast, in gray matter, oligodendrocytic Cx32 forms heterotypic GJ channels with astrocytes by Cx32/Cx30 channels, where it is additionally expressed mainly in perikarya and proximal processes (Orthmann-Murphy et al. 2008). Distinctly different distributions of Cx32 and Cx29 are also observed in the PNS throughout the myelinating Schwann cells (Ahn et al. 2008; Altevogt et al. 2002; Li et al. 2002). Cx32/Cx30 GJCs are mainly found on the outer layer of the myelin sheaths and on oligodendrocyte somatic regions in the preferentially gray matter. Still, they are also observed in less number in the white matter regions. In white matter regions, Cx47/Cx43 channels are primarily observed.

Myelinating Schwann cells in the PNS do not take part in forming GJICs with the neighboring cells but rather form "reflexive" intracellular GJs connecting different regions within the same cell (Menichella et al. 2003). These reflexive junctions, present at the paranodal cell membranes and Schmidt-Lanterman incisures, help retain cytoplasm and help in providing continuity between the perinuclear and periaxonal cytoplasm in the PNS (Scherer et al. 1995). Both Cx29 and Cx32 are important in the PNS.

As the inter-oligodendrocytic GJs appear to be concentrated at paranodes, and oligodendrocytes are dependent on astrocytes for small molecule homeostasis, the astrocyte/oligodendrocyte GJ coupling is more crucial near oligodendrocytic somata and proximal processes. Cx43/Cx47-mediated channels mainly form the astrocyte/oligodendrocyte GJs, and Cx43/Cx47 outnumbers Cx30/Cx32 channels at the oligodendrocyte cell bodies (Orthmann-Murphy et al. 2008; Wasseff and Scherer 2011). At the subcellular level, another α-Cx, Cx47 (GJA12), is prominent in oligodendrocyte somata and proximal processes, in a "beads-on-a-string" fashion as well as through the outer layer of the myelin sheath present in both the white and gray matter of the CNS, and forms GJICs with the astrocytic processes. In mice, Cx47 is only found in myelinating cells in the CNS but not in Schwann cells in PNS.

Cx47, which is expressed entirely by oligodendrocytes (previously believed to be by neurons) (Teubner et al. 2001), is mainly observed most abundantly but not exclusively in cells of white matter region like the deep cerebellar white matter, corpus callosum, spinal cord white matter, and optic nerve (Odermatt et al. 2003). Large numbers of Cx47-positive cells are also seen in the anterior commissure, the optic chiasm, and the striatum. This GJ network plays a crucial role in distributing the excess K+ ions and glutamate during neuronal activity and putatively also provides a lactate shuttle and mediates the propagation of ICWs.

The neurons mainly express Cx36, which forms GJICs between the neuron only in the adult brain. Cx36 expression is mainly observed in hippocampal interneurons, olivary nucleus, and cone photoreceptor cells. Neurons are also reported to express Cx45 and Cx57, but the expression of these Cxs varies spatiotemporally and depends on the type of neurons (Sohl et al. 2005). Brain fibroblasts express mainly Cx43 and Cx26 in the leptomeningeal layer (Spray et al. 1991).

2 Gap Junction Protein Mutations in Health and Disease

In humans, mutation of Cx genes causes different diseases, and CNS pathology is associated with some of them. The mutation in the human Cx43 gene (GJA 1), which is located at human chromosome 6q22–q23, is reported to cause oculodentodigital dysplasia (ODDD). ODDD patients manifest craniofacial (ocular, nasal, and dental) and limb abnormalities, spastic paraplegia, microcephaly, and neurodegeneration. The disease is also characterized by malfunctioning of the cardiac tissue. As the name suggests, the disease is also associated with abnormal primary and permanent dentition, hypoplasia or aplasia of the middle phalanges, and ophthalmic malformations including microphthalmia, cornea and iris abnormalities, and optic atrophy. ODDD symptoms include neurologic disease phenotypes like demyelinating diseases (dysarthria, neurogenic bladder, ataxia, muscle weakness, spasticity) and seizures (Loddenkemper et al. 2002). Mild mental retardation also occurs infrequently. Brain magnetic resonance imaging (MRI) studies of ODDD patients have demonstrated diffuse bilateral abnormalities in the subcortical cerebral white matter regions, which defines a progressive leukodystrophy. Hence, functional Cx43 expression is crucial for CNS myelination. It is concluded that the complex combinatorial interactions exerted by Cx43 help in the maintenance of CNS myelin (Paznekas et al. 2003).

The neurological phenotype in ODDD is linked to aberrant Cx43 channels. Atrophy of the optic nerve results in the loss of visual function (a reduction in normal sharp vision) or blindness, like the loss of visual acuity observed during neuromyelitis optica (NMO). The physiology of Cx43 mutation is partially reproduced in astrocyte-specific Cx43 knockout mice which exhibit retarded motor performance (Frisch et al. 2003), like ODDD patients with symptoms of cerebellar ataxia.

The previously described disease models elucidate the role of astrocyte/astrocyte Cx43/Cx43 and astrocyte/oligodendrocyte Cx43/Cx47 GJCs in K+ buffering and

nutrient homeostasis in CNS. Thus, the redistribution of ions and small molecules might affect neurotransmitter uptake/release and maintain and maintain osmotic balance, which affects the pathology in ODDD. It is also observed that the retention of monomeric/oligomeric Cx43 protein can induce glial cell death through unfolded protein response (UPR) or endoplasmic reticulum-associated protein degradation (ERAD) (Roussel et al. 2013). The ER stress can lead to depletion of GJ expression as well. The UPR and ER stress itself has been observed in different neurodegenerative diseases (e.g., Huntington's disease, HD; amyotrophic lateral sclerosis, ALS), also observed in ODDD phenotype. It is important to note that in humans, one dysfunctional allele of Cx43 may lead to a CNS phenotype observed in ODDD. In contrast, homozygous astrocyte-specific Cx43 knockout mice exhibit no severe alterations (Theis et al. 2003). Hence, specifically in humans, ODDD pathology portrays a dominant-negative phenotypic effect of mutant Cx43. In humans, though astrocyte/oligodendrocyte GJIC is also mediated through Cx30/Cx32 channels, these GJICs do not appear to compensate for the loss of Cx43/Cx47 GJCs, probably due to differences in conductance, gating, and permeability of the two types of heterotypic channels (De Bock et al. 2013).

In the demyelinating lesions of patients with Baló's disease (a disorder characterized by astrocytopathy and demyelination), a severe reduction of astrocytic Cx43 is observed (Masaki et al. 2012). This finding adds to the hypothesis that aberrant expression and function of astrocytic Cx43 are linked to aberrant axonal myelination.

The Pelizaeus-Merzbacher disease (PMD) is characterized by nystagmus, ataxia, abnormal motor functions, dysarthria, and progressive spasticity. PMD is caused by Plp1 gene mutation. This gene encodes for proteolipid protein 1 (PLP1). PLP1 is one of the significant components of CNS myelin and is also expressed in PNS myelin. But not an only mutation of Plp1, but mutation of gap junction protein also causes a similar disease phenotype.

Like PMD, the Pelizaeus-Merzbacher-like disease (PMLD) is caused by mutation of the *GJA12* gene, which encodes for Cx47, and oligodendrocytic GJ partner of astrocytic Cx43. Both diseases show hypomyelination and leukodystrophy in the CNS, which dramatically resembles the neuropathology of ODDD (Uhlenberg et al. 2004). However, a few key features like the slower progression of PMLD symptoms, preservation of cognitive functions, and partial myelination of corticospinal tracts diagnosed by MRI differentiate the pathological hallmarks of PMD and PMLD. Overall, the mutations of PLP1 and Cx47 exert similar pathology, which is additional proof that these genes are involved similarly in regulating CNS myelin function in humans. Similar to the ablation of Cx43 in the murine model, Cx47 knockout animals do not exhibit severe pathological features (Odermatt et al. 2003).

In contrast, the patients affected due to PMLD have missense mutations on one or both alleles of the gene, resulting in the synthesis of mutant Cx47 protein, which might have a dominant-negative effect on the entire GJ repertoire. The phenotype of PMLD might depend on mutant gene dosage. Hence, the cellular mechanisms exerted by the mutant Cx47 proteins may lead to hypomyelination leukodystrophy

Table 1 Genetic disorders linked to dysmyelination and similar diseases

Etiology	Disease name	Pathology associated with CNS/PNS
Mutation of Cx43	Oculodentodigital dysplasia (ODDD)	Some of the cases exhibit optic atrophy, dysfunction of CNS myelin, and progressive leukodystrophy
Mutation of PLP1	Pelizaeus-Merzbacher disease (PMD)	Nystagmus, impaired motor development, and leukodystrophy
Mutation of Cx47	Pelizaeus-Merzbacher-like disease (PMLD-1)	Hypomyelination leukoencephalopathy
Mutation of peripheral myelin protein 22 (PMP 22)	Charcot-Marie-Tooth disease (CMT)	Motor and sensory neuropathies, peripheral dysmyelination
Mutation of Cx32	Charcot-Marie-Tooth disease type 1 (CMTX 1)	Mainly peripheral demyelinating disorder, with a few of them showing CNS hypomyelination

in PMLD. Table 1 summarizes the CNS and PNS demyelinating/dysmyelinating diseases we discussed primarily in this chapter.

Alteration of other Cxs is also linked to different human diseases. For example, Cx46 and Cx50 mutations cause cataracts, and most of the Cx26, Cx30, and Cx31 mutants cause loss of auditory function or dominantly inherited hearing loss and skin diseases like hidrotic ectodermal dysplasia. The missense mutations in Cx26 were found to be associated with palmar and plantar keratoderma with sensorineural deafness. Mutations in Cx31 and Cx30.3 can also cause erythrokeratodermia variabilis (Orthmann-Murphy et al. 2007).

3 The Cx43/Cx47 Axis in CNS Myelination

The formation and maintenance of myelin about GJ function are an open field in cell biology and neurobiological research. Cx32 (*GJB1*) is crucial for peripheral myelination. Interestingly, the mutations in the gene *Gjb1*, encoding for Cx32 protein, cause a primarily peripheral demyelinating neuropathy named X-linked Charcot-Marie-Tooth disease type 1 (CMTX1) (Bergoffen et al. 1993). Cx32-mediated GJIC has been implicated in providing a pathway for the lateral diffusion of molecules directly across the peripheral myelin sheath formed by Schwann cells. More than 200 different Cx32 mutations have been found to cause CMTX1, but only a few of these mutations are associated with rare CNS abnormalities (Kleopa and Scherer 2002). The CNS manifestations in CMTX1 are only observed under metabolic stress conditions and inflammation or over the later human lifespan. Hence, CMTX1 pathology is mainly restricted to PNS, indicating that astrocyte/oligodendrocyte Cx30/Cx32 coupling is not critical for human CNS myelination. It is also noteworthy that in humans, Cx47 expression is present in both CNS and PNS, whereas mice express Cx47 only in CNS oligodendrocytes, which contributes to

GJCs within panglial networks. CNS pathological manifestations are observed in a subpopulation of patients with CMTX1. Importantly, these CNS pathologies are transient or milder than that of PMLD phenotypes. Consistent with this, *Gjb1* knockout mice do not exhibit altered myelination in CNS neural tracts (Scherer et al. 1998). Vice versa, mutations of Cx47, which exert a PMLD phenotype, are also associated with a mild peripheral neuropathy (Uhlenberg et al. 2004). Several case studies and experiments underline the concept that Cx43/Cx47 GJIC is critical for human CNS myelination. Although, in mice, these channels appear to be partially replenished by Cx30/Cx32 channels, in humans, Cx43/Cx47-mediated GJIC is critically important for the formation and maintenance of CNS myelin. This might be due to unique properties possessed by Cx43/Cx47 channels, compared to Cx30/Cx32 channels, in terms of GJIC activity and localization. These observations raise the question, whether the myelination processes are fundamentally different in CNS and PNS in terms of the requirement for functional GJCs, requiring further investigation.

4 Gap Junctions in Human CNS Demyelinating Disease Multiple Sclerosis

As described in the previous sections, Cxs are crucial for human myelination. Multiple sclerosis (MS) is a human CNS demyelinating disease characterized by inflammatory foci in the CNS leading to demyelination, which is associated with concurrent axonal loss and reactive astrogliosis. Although immunomodulatory therapies may be partially successful in disease protection in the relapsing-remitting phase, reducing the frequency and spread of demyelinated plaques, secondary progression and concurrent neurodegeneration are current potential challenges to the currently available therapies, mainly targeting the peripheral immune process. Despite the oligodendrocyte precursor cells (OPCs) being recruited to the focal lesions formed due to loss of myelin and myelin maintaining oligodendrocytes, remyelination is often incomplete. Oligodendrocytic GJs are vital for the generation and maintenance of CNS myelin, but their involvement in MS progression is relatively unexplored. The alteration of GJs during MS and its role in disease progression is another crucial focus of this review.

GJs are vital for CNS myelination, but how GJs are altered and their role in MS is only recently being investigated. An important study performed by Markoullis et al. (2012b) elucidates the alteration of GJs in MS brains, which is dissected into three different types of white matter: normal appearing white matter (NAWM), chronic active lesions, and inactive lesions. Beyond focal lesions, cell biological aspects of the remodeled environment in the NAWM appear to be critical for mediating disease progression, including specific pathological signatures like microglial activation, axonal degeneration, and disruption of BBB. The identification of mechanisms, which lead to expansion of chronic demyelinated plaques and axonal loss in both MS lesions and NAWM, is primarily important for developing novel therapeutic targets in SP-MS. The impact of altered homeostasis in neuroinflammatory condition

as well as how alteration of GJ network contributes to the MS progression and pathology remains mostly unexplored. However, recent research elucidates that the maintenance of panglial network connectivity portrays a crucial role in restricting MS pathology and promoting repair. The expression of astrocytic Cx43 is elevated in active MS lesions, significantly diminished in inactive lesions and is not significantly altered in NAWM (Markoullis et al. 2012b).

A study on MS patient samples demonstrated that increased Cx43 immunoreactivity was more profound around activated astrocytes. Double immunostaining for GFAP and Cx43 showed intensely GFAP-expressing cells also express profuse amounts of Cx43 immunoreactivity within lesions, indicating that astrogliosis in lesions led to higher Cx43 expression. In contrast, oligodendrocytic Cx47 mRNA levels remained significantly depleted both in lesions and in NAWM. Immunofluorescence studies and GJ plaque counts showed that Cx47 was non-significantly increased in MS NAWM compared to normal healthy brains but counts of Cx47 plaques progressively reduced toward the lesions. In addition, Western blot studies demonstrated a non-significant upregulation of Cx47 in MS NAWM and a significant depletion of Cx47 in MS lesions. This non-significant increase in Cx47 was associated with OPC activity. The upregulation of Cx43 protein levels, may alter in GJ plaque counts, GJCs formation, and HC formation, as a consequence of glial scar formation which causes pathological alteration of astrocytes. The upregulation of Cx43 was also associated with upregulation of CD11b, a microglia/macrophage marker, expressed in high amounts around the demyelinated plaques (Markoullis et al. 2012b).

It was seen that OPCs are recruited to chronic MS lesions (Reynolds et al. 2002). In this case, OPC numbers increased in MS NAWM compared to a normal person's white matter, but fewer mature oligodendrocytes (Olig2-negative) were present. In the NAWM, these OPCs were observed to express Cx47, while in normal white matter, OPCs do not express Cx47 in general. Furthermore, Cx43 staining was mainly concentrated between the activated astrocytes, but Cx47 and Cx43 GJ colocalization was reduced. Although it was previously believed that OPCs do not participate in GJ communication, only mature oligodendrocytes have detectable GJ connectivity. Recently, it has been shown that OPCs might be functionally coupled to white matter oligodendrocytes.

Furthermore, partial loss of the OPCs has been observed due to the conditional deletion of astrocytic Cx43 (Maglione et al. 2010). Thus, Cx43 expression is important for OPC proliferation and differentiation, and OPCs may be connected to panglial network by GJ communication. The ability of Cx43 to confer adequate GJ connectivity might affect functional oligodendrocyte maturation in demyelinated areas and promotion of remyelination in MS. Whether this phenomenon influences disease progression remains to be evaluated.

Double immunofluorescence for OPCs/mature oligodendrocytes and Cx47 revealed that, in MS NAWM, OPCs demonstrated increment in Cx47-positive puncta, but fewer and smaller GJ plaques were observed in mature oligodendrocytes. The elevated Cx47 expression in OPCs was associated with increased numbers of these cells recruited to repair MS lesions. In contrast, non-MS white matter OPCs

were mostly not expressing Cx47. Coimmunostaining of an OPC marker and astrocytic Cx43 showed that OPCs were surrounded by less number of Cx43 GJ plaques. GJs were reduced along myelinated fibers of matured oligodendrocytes. Interestingly, as a part of myelin repair, recruited OPCs upregulate GJ expression, which appears to establish metabolic connectivity to the surrounding astrocytes. Due to astrogliosis and pathological alterations in astrocyte phenotype, Cx43/Cx47-mediated GJ coupling became partially re-established. Thus, OPC connectivity to astrocytes is predicted to be limited in NAWM of MS patients. As Cx47 is primarily localized in oligodendrocytic somata and proximal processes, the loss of oligodendrocytic Cx47 was predicted to be directly associated with demyelination and loss of oligodendrocytes. Another oligodendrocytic GJ, Cx32, mainly located in oligodendrocytic somata in gray matter, establishes GJ communication with oligodendrocytes. Cx32 present along large, myelinated fibers in white matter was reduced in and around MS lesions as well as in the NAWM. Loss of Cx32 GJs in the myelin sheath may be a contributing factor, which impairs panglial signaling and disrupts the radial spread of ICWs, and second messengers impair ionic homeostasis and deplete glucose/metabolite delivery. As only the oligodendrocytes but not OPCs express Cx32, a significant loss of oligodendrocytic Cx32 is observed in NAWM, in contrast to Cx47.

The contrasting patterns between Cx47 (which reduces more toward the plaques) and Cx43 (which increases more toward the plaques) expression in MS-affected brains and reduced colocalization between the two Cxs lead to hypothesize that the disruption of Cx43/Cx47-mediated astrocyte/oligodendrocytic GJ coupling may play a critical role by which demyelinated lesions slowly expand during SP-MS. This is mainly caused by dysfunction of the panglial GJ network and withdrawal of homeostatic and metabolic support to the oligodendrocytes. This initial Cx43 loss was predicted to disrupting most astrocyte/oligodendrocyte GJ connections. Cx47 was reported to be stabilized by Cx43 in vivo. Hence, during chronic demyelination and astrogliosis, Cx43 was replenished back to its normal level, but the loss of Cx47 did not recover. Notably, the myelin marker PLP expression in mRNA level in the NAWM was similar, but PLP expression was found to be significantly diminished in the inactive lesions. Inactive MS lesions PLP was reduced, but the extent of reduction was comparatively less prominent. Earlier studies showed that OPCs rarely express GJs and only mature O10+ oligodendrocytes take part in the GJ network in the panglial system. However, this concept is changed significantly by current research. During MS, the OPCs that express Cx47 continue during their differentiation to establish communication with the glial syncytium. This might be an essential factor in OPC maturation and their ability to remyelinate.

In contrast, loss of GJ connectivity in mature oligodendrocytes appears to be a contributing factor to the expansion of focal lesions. Thus, whether the ability of OPCs to establish GJ communication to the panglial network affects the process of remyelination and influences disease progression warrants further investigation (Markoullis et al. 2012b).

5 Remodeling of Gap Junction Proteins in Experimental Animal Models of Multiple Sclerosis

The interaction between the immune system (both innate and adaptive) and the CNS makes the mechanistic understanding of MS complex. MS, a disease whose etiology is yet fully understanding, requires developing animal models to address its pathogenesis. Several animal models of demyelination are now used to unravel the key mechanism of MS, including immune-mediated, toxic, viral, and genetic pathogenesis.

An autoimmune model, experimental autoimmune encephalomyelitis (EAE), is the most widely used model in MS research. EAE models have been used to understand the role of GJ communication during MS. In EAE, upregulation of astrocytic Cx43 and loss of oligodendrocyte GJs are observed in chronic EAE stages (Roscoe et al. 2007). An initial study showed the downregulation of Cx43 in the acutely inflamed spinal cord in EAE, propagated by adoptive transfer of T cells, whereas the consequences on oligodendrocyte Cxs remained unknown. This study showed spinal cord white matter was massively infiltrated with CD11b-positive macrophages. Cx43 immunoreactivity was depleted in macrophage-infiltrated areas. Astrocytes were strongly GFAP-positive in these Cx43-depleted lesions. Notably, swelling of astrocytes was observed to precede macrophage infiltration in the CNS. Spinal cord tissues, harvested at the peak of the acute phase of the disease, showed significantly reduced *Cx43* RNA expression. Immunohistochemical data supported the decrease in Cx43 in distinct regions of the white matter, although the exact mechanism was not explained. The authors hypothesized that the membrane-associated factors and soluble immune molecules like IL-1β likely mediated the decrease in astrocytic Cx43. The inflamed regions showed diminished Cx43 expression, which was not compensated by the expression of Cx30. So, in the acute phase, loss of major astrocytic Cxs was predicted to minimize the spread of damage mediated by bystander effect. Still, it was also hypothesized as a significant contributing factor in the inflammation-associated disruption of homeostasis (Brand-Schieber et al. 2005).

In later studies, the reduction of Cx43 observed in acute EAE was hypothesized to induce the disruption of astrocyte/oligodendrocyte GJ coupling, which was sustained till the chronic stage of the disease. In acute EAE, Cx43 GJ plaques were dramatically depleted within and around the inflamed region of the white matter, where GFAP-stained astrocytic processes were observed, but they were devoid of Cx43. Cx43 immunoreactivity increased within lesions above normal levels during the chronic relapsing phase and colocalized with dense GFAP immunoreactivity (which reflected astrogliosis). *Cx43* expression was reduced in mRNA level at early EAE stages. Oligodendrocytic *Cx* mRNAs were marginally or not at all downregulated. Neither increased degradation nor reduced expression of Cx47 was observed during acute EAE. Western blots of the Cx protein levels supported that Cx47 levels were similar to normal levels in EAE mice during the acute phase, suggesting that Cx47 localization only is altered. Cx47 immunoreactivity is observed away from the cell membrane, and it diffuses intracellularly. Importantly,

in vivo, Cx43 controls Cx47 stability in GJ plaques. Thus, the retention of Cx47 in an intracellular compartment might be caused by the loss of astrocytic Cx43 during acute EAE. The reduction of Cx43-mediated GJ plaque numbers in the acute phase, which was seen to be later increased from the NAWM to the perilesional areas and to the lesions of the MS patients, might only reduce functional stability of oligodendrocytic GJs in heterotypic plaques during the onset and acute phase of disease. In contrast, in the chronic phase, a reverse gradient of Cx43 expression was found, where Cx43 was increased from NAWM to the perilesional areas and to the lesions. Cx43 protein levels and GJs were marginally elevated during chronic active inflammation. At later stages, Cx47 expression dropped and remained depleted although Cx43 expression increased. This time, Cx47 and Cx32 were persistently lost, even away from lesions and Cx47 (Markoullis et al. 2012a).

GJ plaques were significantly depleted inside and around EAE lesions, with diffused Cx47 immunoreactivity, which was detectable in the cytoplasm of oligodendrocytes. At the time of disease remittance, remyelination was associated with an increased number of Cx47-mediated GJ plaques. However, during the chronic demyelinating phase, there was a further depletion of Cx47 and Cx32 proteins observed throughout the spinal cord white matter. As the ratio of GJ plaques per oligodendrocyte was significantly reduced, it was not predicted that oligodendrocyte death caused Cx47 downregulation; instead, it was predicted that Cx43-mediated stabilization of Cx47 was important. During remyelination, OPCs showed only restricted communication to the astrocytes. In brief, the loss of Cx47 GJ plaque during acute EAE coincided with the loss of astrocytic Cx43, but at later stages, upregulation of Cx43 expression only induced the partial recovery of oligodendrocytic Cx47. Astrogliosis was predicted to increase the number of Cx43 plaques inside lesions. It was demonstrated that only GFAP-positive astrocytes formed homotypic Cx43/Cx43 channels but not heterotypic Cx43/Cx47 channels with oligodendrocytes (Markoullis et al. 2012a, 2014).

During acute EAE, Cx32 was depleted in long myelinated tracts and NAWM, where myelin morphology was normal. Upon genetic ablation of the Cx32 gene (Cx32 knockout), EAE was exacerbated. These data emphasize that the loss of Cx32 also critically contributes to the disruption of ionic homeostasis, intercellular ICW propagation, and metabolic support. The loss of Cx32 in both the demyelinated area and NAWM demonstrates that the loss of GJ network might occur before pathological alteration, but it is sustained until the point of inflammatory demyelination (Markoullis et al. 2012a).

In summary, during the acute stage of the autoimmune model of MS, loss of astrocytic Cx43 induces intracellular localization of Cx47. During disease progression in EAE, oligodendrocyte Cx47 expression increases as a part of remyelination but is never replenished back to its normal level. Cx43 expression is upregulated during lesion formation as a part of reactive gliosis, but Cx47 expression is not replenished back to its normal level. Thus, persistent loss of Cx43/Cx47 GJCs emerges as a noticeable phenomenon and is directly associated with both EAE and MS pathology. Although disruption of ionic and metabolic homeostasis is hypothesized to contribute critically, which same molecules are involved in this is yet to be pinpointed.

6 Alteration of Cx43/Cx47 Axis in Respect to Viral Model of Multiple Sclerosis

Although EAE model of MS is the most widely used model of MS, this model is limited to shed light on the initial involvement of CNS resident glial cells. EAE model also poorly elucidates the initial mechanism of CNS restricted inflammation (neuroinflammation), which might be independent of involvement of the peripheral immune system. In addition, current understanding of MS pathobiology emphasizes the infectious etiology. A significant number of MS patients show high concentrations of IgG in the CSF and brain, named oligoclonal bands. Although, till date, no replicating virus has been isolated from a demyelinating lesion from CNS of a MS patient but there are multiple viruses in animal systems that induces demyelination. The examples include infection in a murine system with Theiler's murine encephalomyelitis virus (TMEV) or a few neurotropic strains of mouse hepatitis virus (MHV), dogs with canine distemper virus, etc. However, the TMEV-induced model of MS elucidates little information on the alteration of metabolic coupling. A viral model of MS, induced by neurotropic mouse hepatitis virus (MHV), MHV-A59, or its recombinant strain RSA59, successfully unraveled the involvement of CNS resident cells, which are important to initiate neuroinflammation during MS. The neurotropic strain of MHV, MHV-A59, induces a biphasic disease. Hepatitis and meningoencephalitis are observed in the acute phase of infection (days 5–6 p.i.), and chronic demyelination and axonal loss are observed in the chronic phase of the disease (day 30 p.i.). Panel A (adapted from Das Sarma (2010)) of Fig. 1 demonstrates the acute and chronic phase pathologies in MHV-A59 infection. In the chronic phase, we observed hypomyelinated areas caused by MHV-A59 infection in the chronic phase (Fig. 1b), which was associated with disruption of normal perikaryonic distribution of Cx47 (Fig. 1c).

It is shown that MHV-A59 infects neurons and other glial cells like oligodendrocytes, microglia, and astrocytes (Chatterjee et al. 2013; Das Sarma et al. 2008, 2009; Kenyon et al. 2015). Its infection of neurons is predicted to be causing axonal degeneration and axonal loss. The resident immune cells in CNS, microglia, are activated and produce inflammatory cytokines. Microglia are observed to strip off dead-sick myelin, which is proposed to be a major cause of demyelination. Importantly, this model elucidates the role of the innate immune system in the primary disease progression in MS, which was a long-standing question in this field. Oligodendrocytes and astrocytes are also directly infected by this virus. Previously, it was found that oligodendrocytes and, more importantly, astrocytes act as a viral reservoir leading to viral persistence.

In contrast, how astrocytes react upon infection and their role in disease progression were largely unknown. Astrocytes, the most abundant glial cell in CNS, control CNS homeostasis and help in ionic and nutrient buffering. Gap junctions, mediated by Cx43, play a major role in this perspective, discussed in earlier sections.

Our studies investigated whether the neurotropic demyelinating strain of MHV, MHV-A59, alters Cx43 expression and is linked to CNS demyelinating pathology in this viral model of MS. In primary astrocyte culture, Cx43 is observed to be reduced

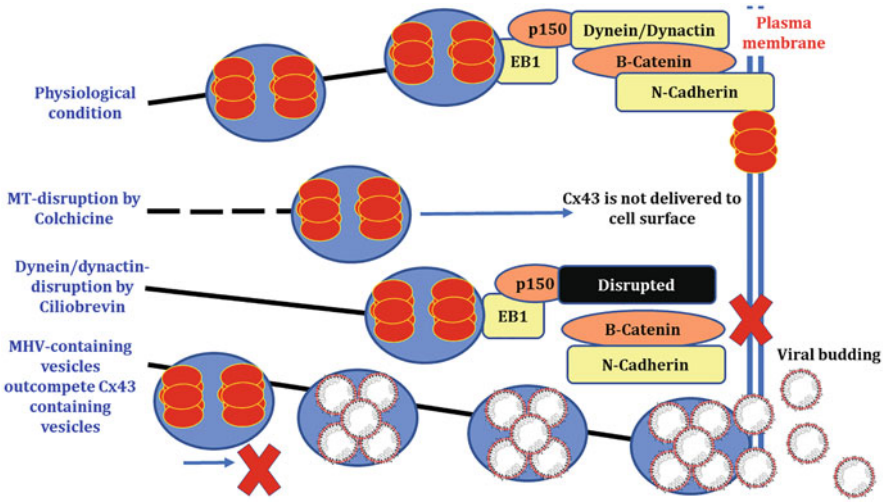

Fig. 1 Acute and chronic stage pathologies induced by MHV-A59 infection in mice. Panel A shows the acute stage infection of MHV-A59 that causes hepatitis, meningitis, optic neuritis, and neuroinflammation which results in myelitis, demyelination, and axonal loss in chronic phase. This chronic stage pathology is also reflected in putative hypomyelinated areas in mouse brain (arrow, Panel B). The higher magnification images show the abnormalities in myelinated tracts (stained for PLP) were associated with disruption of perikaryonic stain of Cx47, which is evident in MHV-A59-infected brains but not in mock-inoculated brains (Panel C)

in both mRNA and protein levels. In addition, a substantial amount of translated Cx43 was retained in the ER/ERGIC intracellular compartment (Basu et al. 2015). Previously, it was shown that few ODDD mutations also showed intracellular localization pattern of Cx43, which was mainly colocalizing with ER marker PDI and Golgi marker Giantin or TGN-38. The colocalization was mainly observed with ER markers and minimal with Golgi markers (Gong et al. 2006). Our model showed that virus infection directly leads to similar ER/ERGIC localization, affecting functional channel formation on the cell surface. Indeed, these phenomena led to a severe loss of GJ plaque formation between astrocytes and significantly reduced the functional channel formation between astrocytes. Retention of GJs in ER/ERGIC is the most significant phenomenon, as observed in other models of MS, but the direct cause-effect relationship was unknown. As discussed, both Cx43 and viral particles use MTs to reach the cell surface. Hence, we further investigated whether MT-mediated viral trafficking was one of the major causes restricting Cx43-mediated GJ plaque formation on the cell surface. It was observed that Cx43/MT interaction was directly affected due to the interaction between viral particles/MT and viral particles replaced Cx43 near the cell surface (Basu et al. 2017). This phenomenon is diagrammatically represented in Fig. 2 and Fig. 3.

Along with that, our group demonstrated the loss of Cx43 was limited to not only astrocytes but also Cx43-expressing blood-brain barrier cells, i.e., meningeal fibroblasts, due to MHV (demyelinating strain) infection (Bose et al. 2018).

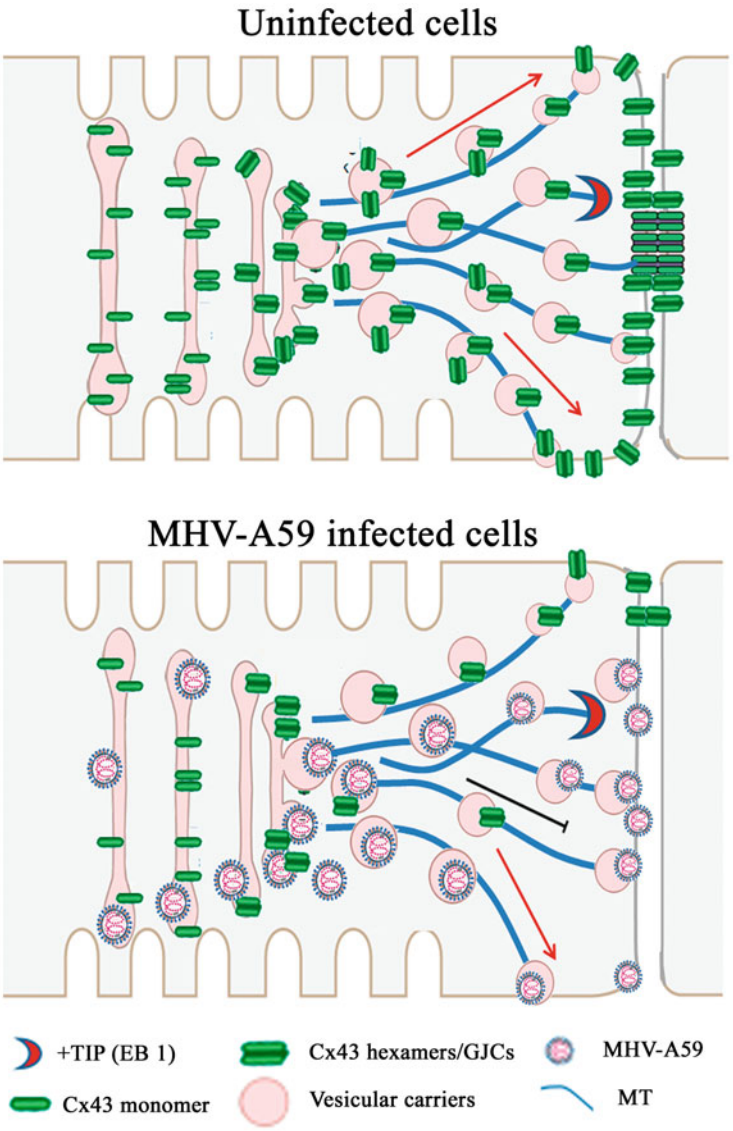

Fig. 2 Physiological role of microtubule (MT) network and associated adapter proteins in Cx43 trafficking to cell surface. MT network helps in the delivery of Cx43 containing vesicles with the help of other proteins like EB1, p150 (glued), dynein/dynein complex, β-catenin, and N-cadherin where Cx43 directly interacts with MT network demonstrated by immunoprecipitation. Upon chemical-induced disruption of MT network by colchicine and dynein/dynactin complex by ciliobrevin, we observed Cx43 is not delivered to cell surface. During MHV infection, similarly Cx43 is retained inside the cells as the virus-containing vesicles outcompete Cx43-containing vesicles. However, which complexes help in the delivery of MHV-containing vesicles is not completely understood

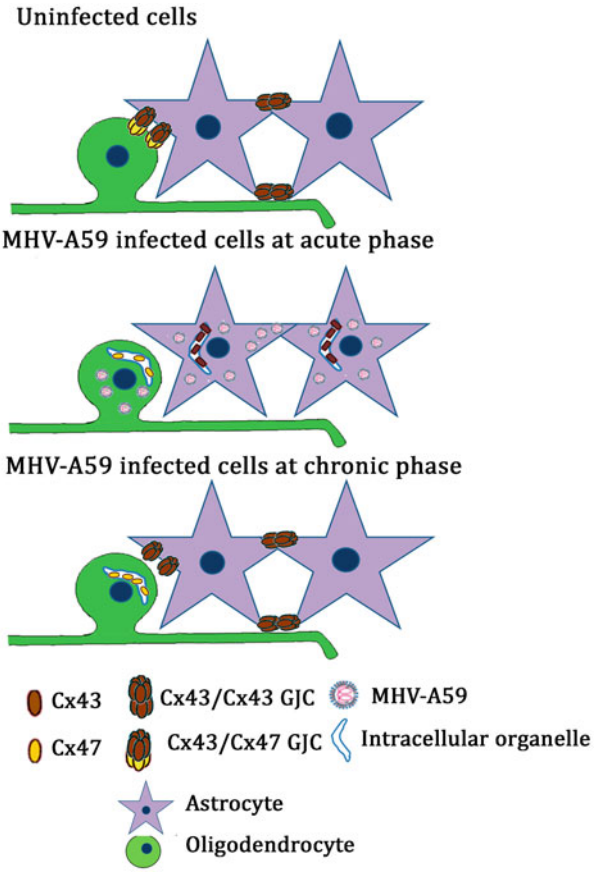

Fig. 3 Microtubule-dependent trafficking of Cx43 is altered in the presence of MHV-A59. In uninfected primary astrocytes, an abundant amount of Cx43, upon exit from TGN, are packaged in vesicular carriers and delivered to cell surface with the help of MT network. These Cx43 hemichannels then form GJCs and GJPs in the plasma membrane. Upon MHV-A59 infection, viruses emerging in double-membrane vesicles (DMVs) or similar organellar structures dynamically compete out Cx43 to reach to the cell surface, and viruses egress with the help of MT network. There might be competition for MT adaptor protein and cargos, which, in turn, drastically depletes Cx43/MT interaction

As Cx43 found to regulate the stability of Cx47 in heteromeric GJ plaques, it is hypothesized to be a major cause of perturbed GJ communication and altered homeostasis in vivo. Indeed, depletion of Cx43 expression was observed during acute MHV-A59 infection, which came back to its normal expression level in the chronic demyelinating stage when reproductive viral infection was resolved (Basu et al. 2015). In contrast, oligodendrocytic Cx47 was persistently downregulated from the acute stage of infection to the chronic stage. Notably, during the chronic stage and at the peak of demyelination, loss of Cx47 was associated with loss of myelin marker PLP (Basu et al. 2017). In MS patient samples and other models of MS, altered GJ communication was hypothesized to play a key role, but none of those revealed a mechanistic pathway of initiation of GJ alteration. In a viral model of MS, MHV-A59 infection-induced alteration of Cx43 provides a link to the initial astrocytic Cx43 alteration and ultimately observed altered astrocyte/oligodendrocyte GJ

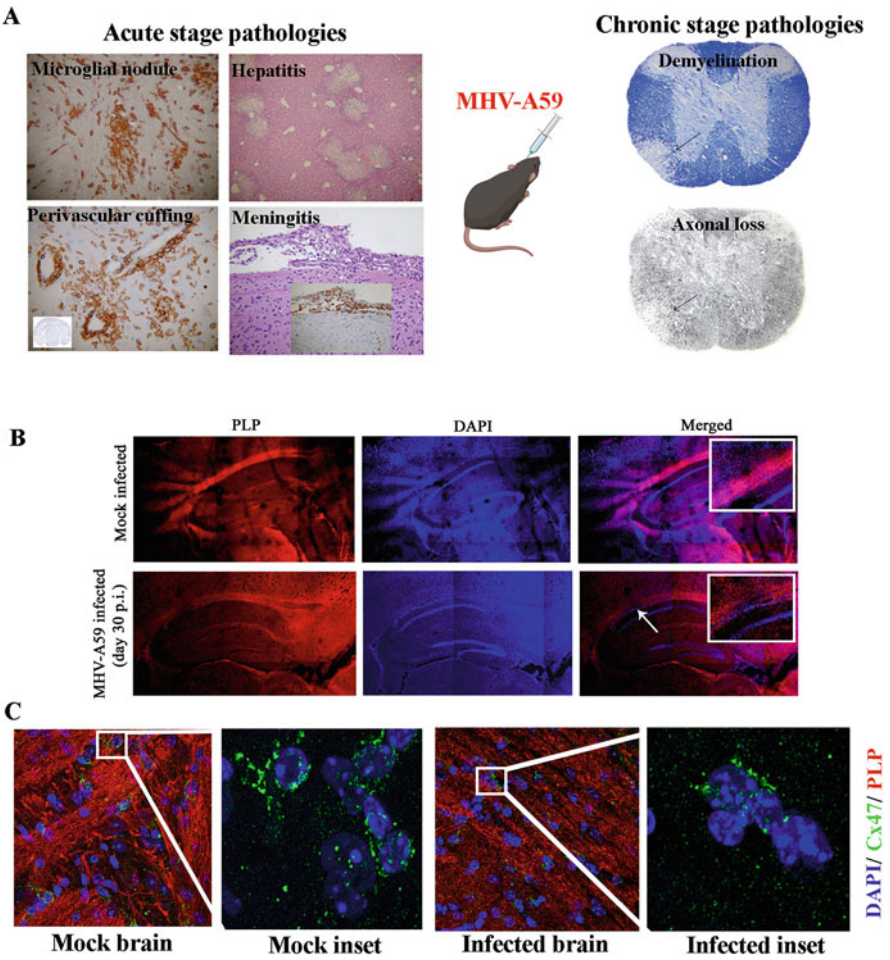

Fig. 4 Probable model of alteration of astrocyte/oligodendrocyte Cx43/Cx47-mediated GJC in MHV-A59 infection and neuroinflammation. Uninfected astrocytes and oligodendrocytes maintain GJIC by Cx43/Cx47-mediated channels, and Cx43/Cx43 channels form GJIC between astrocytes. Upon MHV-A59 infection in acute phase, Cx43 expression is downregulated and also retained in intracellular compartment like ER/ERGIC. Cx47 expression is also diminished in acute phase of MHV-A59 infection. During chronic phase of MHV-A59 infection, infectious virus particles are cleared from the system. Cx43/Cx43 homotypic channels start to be replenished back to its normal expression level. Cx47, which is stabilized by Cx43 in vivo, is sustained to be downregulated, and the sustained losses of Cx43/cx47 channels are associated with loss of myelin-specific protein PLP

communication during progressive MS. These observations elucidate its role in perturbed CNS homeostasis leading to loss of CNS myelin. The alterations observed in the Cx43 and Cx47 during MHV-A59 infection in the brain are represented in Fig. 4.

Acknowledgments Supported in part by research grants (BT/PR14260/MED/30/437/2010 and BT/PR4530/MED/30/715/2012) from the Department of Biotechnology, India; research grant (RG3774A2/1) from the National Multiple Sclerosis Society (NMSS), USA; and the Indian Institute of Science Education and Research-Kolkata (IISER-K), India, start-up fund to JDS. The Council for Scientific and Industrial Research (CSIR), India, and Du Pré Grant from the Multiple Sclerosis International Federation (MSIF) provided the research support to RB.

Competing Interests The authors declare that they have no competing interests.

Authors' Contributions RB conceived this review and wrote the initial draft, and JDS critically revised and modified this with the comments and thorough scientific inputs.

References

Ahn M, Lee J, Gustafsson A, Enriquez A, Lancaster E, Sul JY, Haydon PG, Paul DL, Huang Y, Abrams CK et al (2008) Cx29 and Cx32, two connexins expressed by myelinating glia, do not interact and are functionally distinct. J Neurosci Res 86:992–1006

Altevogt BM, Kleopa KA, Postma FR, Scherer SS, Paul DL (2002) Connexin29 is uniquely distributed within myelinating glial cells of the central and peripheral nervous systems. J Neurosci Off J Soc Neurosci 22:6458–6470

Basu R, Banerjee K, Bose A, Das Sarma J (2015) Mouse hepatitis virus infection remodels Connexin43-mediated gap junction intercellular communication in vitro and in vivo. J Virol 90:2586–2599

Basu R, Bose A, Thomas D, Das Sarma J (2017) Microtubule assisted altered trafficking of astrocytic gap junction protein Connexin 43 is associated with depletion of Connexin 47 during mouse hepatitis virus infection. J Biol Chem 292:14747–14763

Bergoffen J, Scherer SS, Wang S, Scott MO, Bone LJ, Paul DL, Chen K, Lensch MW, Chance PF, Fischbeck KH (1993) Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 262:2039–2042

Bose A, Basu R, Maulik M, Das Sarma J (2018) Loss of Cx43-mediated functional gap junction communication in meningeal fibroblasts following mouse hepatitis virus infection. Mol Neurobiol 55:6558–6571

Brand-Schieber E, Werner P, Iacobas DA, Iacobas S, Beelitz M, Lowery SL, Spray DC, Scemes E (2005) Connexin43, the major gap junction protein of astrocytes, is down-regulated in inflamed white matter in an animal model of multiple sclerosis. J Neurosci Res 80:798–808

Chatterjee D, Biswas K, Nag S, Ramachandra SG, Das Sarma J (2013) Microglia play a major role in direct viral-induced demyelination. Clin Dev Immunol 2013:510396

Das Sarma J (2010) A mechanism of virus-induced demyelination. Interdiscip Perspect Infect Dis 2010:109239

Das Sarma J, Iacono K, Gard L, Marek R, Kenyon LC, Koval M, Weiss SR (2008) Demyelinating and nondemyelinating strains of mouse hepatitis virus differ in their neural cell tropism. J Virol 82:5519–5526

Das Sarma J, Kenyon LC, Hingley ST, Shindler KS (2009) Mechanisms of primary axonal damage in a viral model of multiple sclerosis. J Neurosci Off J Soc Neurosci 29:10272–10280

De Bock M, Kerrebrouck M, Wang N, Leybaert L (2013) Neurological manifestations of oculodentodigital dysplasia: a Cx43 channelopathy of the central nervous system? Front Pharmacol 4:5

Dermietzel R, Traub O, Hwang TK, Beyer E, Bennett MV, Spray DC, Willecke K (1989) Differential expression of three gap junction proteins in developing and mature brain tissues. Proc Natl Acad Sci U S A 86:10148–10152

Filippov MA, Hormuzdi SG, Fuchs EC, Monyer H (2003) A reporter allele for investigating connexin 26 gene expression in the mouse brain. Eur J Neurosci 18:3183–3192

Frisch C, Theis M, De Souza Silva MA, Dere E, Sohl G, Teubner B, Namestkova K, Willecke K, Huston JP (2003) Mice with astrocyte-directed inactivation of connexin43 exhibit increased exploratory behaviour, impaired motor capacities, and changes in brain acetylcholine levels. Eur J Neurosci 18:2313–2318

Giaume C, McCarthy KD (1996) Control of gap-junctional communication in astrocytic networks. Trends Neurosci 19:319–325

Gong XQ, Shao Q, Lounsbury CS, Bai D, Laird DW (2006) Functional characterization of a GJA1 frameshift mutation causing oculodentodigital dysplasia and palmoplantar keratoderma. J Biol Chem 281:31801–31811

Iacobas DA, Urban-Maldonado M, Iacobas S, Scemes E, Spray DC (2003) Array analysis of gene expression in connexin-43 null astrocytes. Physiol Genomics 15:177–190

Kenyon LC, Biswas K, Shindler KS, Nabar M, Stout M, Hingley ST, Grinspan JB, Das Sarma J (2015) Gliopathy of demyelinating and non-demyelinating strains of mouse hepatitis virus. Front Cell Neurosci 9:488

Kleopa KA, Scherer SS (2002) Inherited neuropathies. Neurol Clin 20:679–709

Kunzelmann P, Schroder W, Traub O, Steinhauser C, Dermietzel R, Willecke K (1999) Late onset and increasing expression of the gap junction protein connexin30 in adult murine brain and long-term cultured astrocytes. Glia 25:111–119

Li X, Lynn BD, Olson C, Meier C, Davidson KG, Yasumura T, Rash JE, Nagy JI (2002) Connexin29 expression, immunocytochemistry and freeze-fracture replica immunogold labelling (FRIL) in sciatic nerve. Eur J Neurosci 16:795–806

Loddenkemper T, Grote K, Evers S, Oelerich M, Stogbauer F (2002) Neurological manifestations of the oculodentodigital dysplasia syndrome. J Neurol 249:584–595

Maglione M, Tress O, Haas B, Karram K, Trotter J, Willecke K, Kettenmann H (2010) Oligodendrocytes in mouse corpus callosum are coupled via gap junction channels formed by connexin47 and connexin32. Glia 58:1104–1117

Markoullis K, Sargiannidou I, Gardner C, Hadjisavvas A, Reynolds R, Kleopa KA (2012a) Disruption of oligodendrocyte gap junctions in experimental autoimmune encephalomyelitis. Glia 60:1053–1066

Markoullis K, Sargiannidou I, Schiza N, Hadjisavvas A, Roncaroli F, Reynolds R, Kleopa KA (2012b) Gap junction pathology in multiple sclerosis lesions and normal-appearing white matter. Acta Neuropathol 123:873–886

Markoullis K, Sargiannidou I, Schiza N, Roncaroli F, Reynolds R, Kleopa KA (2014) Oligodendrocyte gap junction loss and disconnection from reactive astrocytes in multiple sclerosis gray matter. J Neuropathol Exp Neurol 73:865–879

Masaki K, Suzuki SO, Matsushita T, Yonekawa T, Matsuoka T, Isobe N, Motomura K, Wu XM, Tabira T, Iwaki T et al (2012) Extensive loss of connexins in Balo's disease: evidence for an auto-antibody-independent astrocytopathy via impaired astrocyte-oligodendrocyte/myelin interaction. Acta Neuropathol 123:887–900

Menichella DM, Goodenough DA, Sirkowski E, Scherer SS, Paul DL (2003) Connexins are critical for normal myelination in the CNS. J Neurosci Off J Soc Neurosci 23:5963–5973

Nagy JI, Ionescu AV, Lynn BD, Rash JE (2003) Connexin29 and connexin32 at oligodendrocyte and astrocyte gap junctions and in myelin of the mouse central nervous system. J Comp Neurol 464:356–370

Nagy JI, Li X, Rempel J, Stelmack G, Patel D, Staines WA, Yasumura T, Rash JE (2001) Connexin26 in adult rodent central nervous system: demonstration at astrocytic gap junctions and colocalization with connexin30 and connexin43. J Comp Neurol 441:302–323

Nagy JI, Patel D, Ochalski PA, Stelmack GL (1999) Connexin30 in rodent, cat and human brain: selective expression in gray matter astrocytes, co-localization with connexin43 at gap junctions and late developmental appearance. Neuroscience 88:447–468

Naus CC, Bechberger JF, Zhang Y, Venance L, Yamasaki H, Juneja SC, Kidder GM, Giaume C (1997) Altered gap junctional communication, intercellular signaling, and growth in cultured astrocytes deficient in connexin43. J Neurosci Res 49:528–540

Odermatt B, Wellershaus K, Wallraff A, Seifert G, Degen J, Euwens C, Fuss B, Bussow H, Schilling K, Steinhauser C et al (2003) Connexin 47 (Cx47)-deficient mice with enhanced green fluorescent protein reporter gene reveal predominant oligodendrocytic expression of Cx47 and display vacuolized myelin in the CNS. J Neurosci Off J Soc Neurosci 23:4549–4559

Orthmann-Murphy JL, Abrams CK, Scherer SS (2008) Gap junctions couple astrocytes and oligodendrocytes. J Mol Neurosci 35:101–116

Orthmann-Murphy JL, Enriquez AD, Abrams CK, Scherer SS (2007) Loss-of-function GJA12/Connexin47 mutations cause Pelizaeus-Merzbacher-like disease. Mol Cell Neurosci 34:629–641

Paznekas WA, Boyadjiev SA, Shapiro RE, Daniels O, Wollnik B, Keegan CE, Innis JW, Dinulos MB, Christian C, Hannibal MC et al (2003) Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. Am J Hum Genet 72:408–418

Rash JE, Yasumura T, Dudek FE, Nagy JI (2001) Cell-specific expression of connexins and evidence of restricted gap junctional coupling between glial cells and between neurons. J Neurosci Off J Soc Neurosci 21:1983–2000

Reynolds R, Dawson M, Papadopoulos D, Polito A, Di Bello IC, Pham-Dinh D, Levine J (2002) The response of NG2-expressing oligodendrocyte progenitors to demyelination in MOG-EAE and MS. J Neurocytol 31:523–536

Roscoe WA, Messersmith E, Meyer-Franke A, Wipke B, Karlik SJ (2007) Connexin 43 gap junction proteins are up-regulated in remyelinating spinal cord. J Neurosci Res 85:945–953

Rouach N, Avignone E, Meme W, Koulakoff A, Venance L, Blomstrand F, Giaume C (2002) Gap junctions and connexin expression in the normal and pathological central nervous system. Biol Cell 94:457–475

Roussel BD, Kruppa AJ, Miranda E, Crowther DC, Lomas DA, Marciniak SJ (2013) Endoplasmic reticulum dysfunction in neurological disease. Lancet Neurol 12:105–118

Rozental R, Giaume C, Spray DC (2000) Gap junctions in the nervous system. Brain Res Brain Res Rev 32:11–15

Scherer SS, Deschenes SM, Xu YT, Grinspan JB, Fischbeck KH, Paul DL (1995) Connexin32 is a myelin-related protein in the PNS and CNS. J Neurosci Off J Soc Neurosci 15:8281–8294

Scherer SS, Xu YT, Nelles E, Fischbeck K, Willecke K, Bone LJ (1998) Connexin32-null mice develop demyelinating peripheral neuropathy. Glia 24:8–20

Sohl G, Maxeiner S, Willecke K (2005) Expression and functions of neuronal gap junctions. Nat Rev Neurosci 6:191–200

Spray DC, Moreno AP, Kessler JA, Dermietzel R (1991) Characterization of gap junctions between cultured leptomeningeal cells. Brain Res 568:1–14

Teubner B, Odermatt B, Guldenagel M, Sohl G, Degen J, Bukauskas F, Kronengold J, Verselis VK, Jung YT, Kozak CA et al (2001) Functional expression of the new gap junction gene connexin47 transcribed in mouse brain and spinal cord neurons. J Neurosci Off J Soc Neurosci 21:1117–1126

Theis M, Jauch R, Zhuo L, Speidel D, Wallraff A, Doring B, Frisch C, Sohl G, Teubner B, Euwens C et al (2003) Accelerated hippocampal spreading depression and enhanced locomotory activity in mice with astrocyte-directed inactivation of connexin43. J Neurosci Off J Soc Neurosci 23:766–776

Uhlenberg B, Schuelke M, Ruschendorf F, Ruf N, Kaindl AM, Henneke M, Thiele H, Stoltenburg-Didinger G, Aksu F, Topaloglu H et al (2004) Mutations in the gene encoding gap junction protein alpha 12 (connexin 46.6) cause Pelizaeus-Merzbacher-like disease. Am J Hum Genet 75:251–260

Wasseff SK, Scherer SS (2011) Cx32 and Cx47 mediate oligodendrocyte:astrocyte and oligodendrocyte:oligodendrocyte gap junction coupling. Neurobiol Dis 42:506–513

Generation and Maturation of Macroglia in the Central Nervous System

Nisha Patro and Ishan Patro

Abstract

Nervous system development is a dynamic process that follows a highly constrained and genetically organised pattern leading to the generation of a complex framework for guiding human behaviour. Generation of an appropriate number of neurons and glia at the correct time and location is crucial for the proper development of the neural circuitry and mental functions. The neural epithelial cells (NEPs) undergo a stereotyped programme of cell division to generate both neurons and macroglia through progressive differentiation and commitment. A number of evolutionarily conserved signalling factors direct the progenitors to decide whether to self-renew or generate neurons or various glial cells via activating a specified programme of transcription factor expression in target cells. In the present chapter, we will provide an overview regarding the cellular and molecular basis of the generation of various macroglia (astrocytes, oligodendrocytes and NG2 glia) as well as the developmental mechanisms that direct these cells to acquire specific glial identities in the developing central nervous system. This will strengthen our understanding to explore how these mechanisms can be used to design curative therapeutic strategies for various neurodevelopmental disorders and promote the regeneration and repair of the central nervous system.

Keywords

Neural epithelial cells · Astrocytogenesis · Oligodendrocyte precursor cells · Oligodendrogliogenesis · NG2 glia

N. Patro (✉) · I. Patro
School of Studies in Neuroscience, Jiwaji University, Gwalior, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_6

1 Introduction

The development of the nervous system is a complex, progressive and dynamic process with precise sequence of key events guided by an intricate and well-programmed interplay of intrinsic and extrinsic factors that leads to the generation of a complex framework guiding human behaviour. The human brain is made up of approximately 10^{11} number of neurons and about 4–5 times the number of glial cells. This vast array of neurons and glial cells in the mammalian central nervous system (CNS) is generated through progressive differentiation and commitment from specific progenitor cells based on a strict spatiotemporal pattern. Both neurons and glia (astrocytes, oligodendrocytes, NG2 glia) are generated from the neuroepithelial cells (NEPs) lining the brain ventricles and central canal of the spinal cord. These NEPs act as neural precursor/progenitor cells (NPCs) and generate cells in a strict hierarchal manner, i.e. neurons are produced first followed by glia in an appropriate number, location and timing (Fig. 1). The NPCs are the self-renewable uncommitted multipotent progenitor cells with the capacity to generate a diverse variety of neurons and all the macroglia of the central nervous system (Morrison et al. 1997).

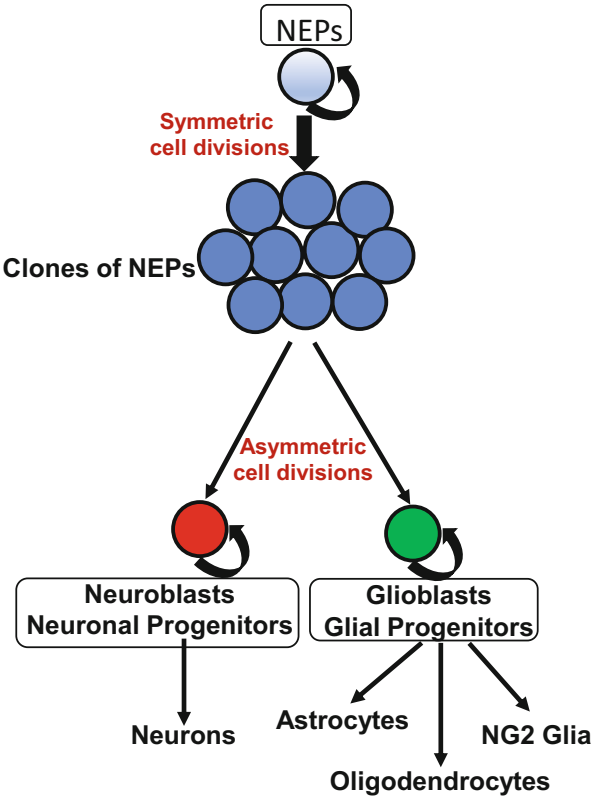

Fig. 1 Hierarchical representation of the generation of neural cells

The transition of fate switch from neuron to glia is temporally regulated by both intrinsic and extrinsic mechanisms that decrease neurogenesis and promote gliogenesis. The differentiation of various neurons and glia is mediated by a small number of signalling factors that control programmes of transcription factor expression in target cells, thus influencing their fate. The lineage history of the cell and the transcription factors it inherits at the time of development determine its responsiveness to these inducing factors. Neurogenesis proceeds in two phases: prenatal phase, when most of the neurons are generated from the ventricular zone (VZ) followed by their migration to their destined locations, and postnatal phase, when only a limited degree of neurogenesis occurs from the subventricular zone (SVZ) targeted to the olfactory bulb and dentate gyrus of the hippocampus. Although the proliferation and migration of glial progenitors start prenatally, the peak astrogenesis is a postnatal event followed by the generation of oligodendrocytes. Most of the neurogenesis and the formation of the basic architecture of the brain is completed during prenatal development, a limited degree of neurogenesis and the generation and maturation of two major glial cells, viz. astrocytes and oligodendrocytes, formation of the synaptic contacts, synaptic pruning and myelination continue for an extended period postnatally (Fig. 2). However, the early brain development is highly pre-programmed and a self-organised temporally constrained process, and failure at any crucial step may lead to catastrophic alterations in brain development. The basic principles of early neural development have been extensively reviewed by several authors (Semple et al. 2013; Stiles and Jernigan 2010; Jiang and Nardelli 2016; Stiles 2017).

In the present chapter, we will provide an overview regarding the cellular and molecular basis of the generation of various macroglia as well as the mechanisms by which these cells acquire specific glial identities in the developing central nervous system. This will further strengthen our understanding to design educational curricula and also how these mechanisms can be used to design interventions for various neuropathologies and promote the regeneration and repair of the central nervous system.

2 Astrocytogenesis

This section provides a brief overview of how and when astrocytes (a) are specified from the neural stem cells (NSCs), (b) acquire morphological and molecular characteristics and cellular heterogeneity and (c) undergo proliferation and functional maturation. Various cortical regions in the vertebrate brain arise mainly from the multipotent progenitor cells in the respective cortical ventricular zones in a strict spatiotemporal manner, and a precise schedule is critical for the organisation of the normal architecture. During the development of the mammalian central nervous system, neurogenesis largely precedes gliogenesis, where neurons are formed first followed by astrocytes and oligodendrocytes. Both neurons and glia originate from the radial glia that act as the primary/apical progenitor or neural stem cells in addition to providing scaffold for the migration of neurons. Neurogenic and

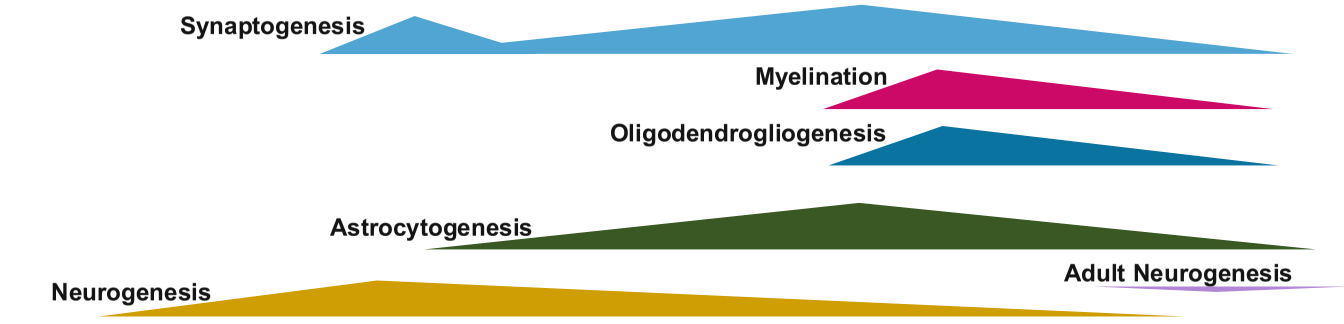

Fig. 2 Schematic timelines of neurogenesis and gliogenesis in rats and humans. The timescale has been adapted from Rice and Barone (2000)

gliogenic capacities of the cortical progenitor cells are influenced by the cell intrinsic abilities as well as extrinsic environmental factors to generate neurons and glia (Qian et al. 2000; Rowitch and Kriegstein 2010; Kang et al. 2012). Fate decisions are controlled by signalling pathways and dynamic transcription factor expressions. In the rodent cortex, the neurogenesis commences around embryonic day (E) 9.5, peaks around E14.5 and finishes around birth (Bayer et al. 1992; Rice and Barone 2000; Babikian et al. 2010; Semple et al. 2013; Patro et al. 2015). Subsequent to the peak neurogenesis, there is a shift from neurogenesis to astrocytogenesis, and the radial glial cells begin to differentiate into glial fibrillary protein (GFAP)-expressing astrocytes (Kriegstein and Alvarez-Byulla 2009; Sanai et al. 2011). The switching of fate from neurogenesis to astrocytogenesis is a key process for generating an appropriate number of neurons and astrocytes and is also crucial for establishing the proper neural circuitry (Freeman 2010; Bronstein et al. 2017).

3 Differentiation and Specification of Astrocytes

The basic understanding of the transcription factors involved in astroglial specification is derived from studies in the developing mammalian spinal cord. The gliogenic switch is a temporally regulated event during which the NSCs from the ventricular zone transit from neurogenesis to gliogenesis. In rodents, this transition occurs around E12.5 in the spinal cord and E16–18 in the cortex (Deneen et al. 2006; Ge et al. 2012). A number of growth factors present in the in vivo environment, viz. epidermal growth factor (EGF), ciliary neurotrophic factor (CNTF), leukaemia inhibitory factor (LIF), platelet-derived growth factor (PDGF), glial growth factor 2 (GGF2) and bone morphogenetic factors (BMPs), have been variously reported to influence this fate switch that suppresses neurogenesis and promotes astrocyte survival, differentiation and maturation over developmental timeline (Qian et al. 2000). How these two processes are coordinated during this switch has been a subject of intense investigation.

The responsiveness of NPCs to these extrinsic signals varies along the developmental timeline. Only the mid-late gestational NPCs are competent to differentiate into astrocytes in response to astrogenic factors. Various astrogenic factors, viz. CNTF, LIF and cardiotrophin-1 (CT-1), have been found to stimulate embryonic NPCs to acquire astrogenic fates by activating the Janus kinase-signal transducer and activator of transcription JAK/STAT pathway (Nakashima et al. 1999; Barnabe-Heider et al. 2005; Takouda et al. 2017). These factors bind to their cognate receptors to induce the dimerisation of gp130 (common signal transducer) or heterodimerisation of gp130 with one of its partner leukaemia inhibitory factor receptor β (LIFRβ) (Ernst and Jenkins 2004). This dimerisation activates JAKs through autophosphorylation and in turn phosphorylates tyrosine residues on the intracellular domain of these receptors where STAT3 is recruited. STAT3 is activated by phosphorylation, forms homodimer and is translocated into the nucleus to bind in the promoter region of the astrocyte-specific genes GFAP and S100β to promote the astrocyte differentiation (Bonni et al. 1997; Nakashima et al. 1999; He

et al. 2005). In addition, BMP signalling also synergistically induces the target genes, where the BMP-activated Smads form a complex with STAT3 via P300/CBP (CREB binding proteins) and induce astrocyte differentiation (Takouda et al. 2017). In the early embryonic NSCs, the *Neurogenin1* (Ngn1) deprives P300/CBP-Smad1, to make complex with STAT3, and thus upregulates the neuronal gene *NeuroD* and inhibits gliogenesis. Ngn expression is downregulated in mid-gestational NSCs; thus, P300/CBP is free to associate with Smad complex of STAT3, leading to a shift from neurogenic to gliogenic fate (Sun et al. 2001; Hirabayashi et al. 2009). Thus, the mechanisms that repress neurogenesis are sufficient to trigger the onset of gliogenesis. Neurogenesis in late embryonic NPCs is also inhibited by notch pathway via transcriptional repressors, such as *HES* genes that prevent the expression of *neurogenins* (Chitnis et al. 1995; Louvi and Artavanis-Tsakonas 2006). Notch signalling is also crucial for gliogenesis and controls gliogenic switch either directly or indirectly by controlling the size of undifferentiated progenitor pool (Androutsellis-Theotokis et al. 2006). In vitro studies have also indicated that in addition to its pro-neural function, Ngn1 also inhibits astrocytogenesis by blocking the JAK/STAT pathway (Ma et al. 1998; Sun et al. 2001; He et al. 2005). Thus, the neuron-glia switch involves complex neuron-glia interactions in a strict spatiotemporal manner involving the downregulation of pro-neural/anti-astrogenic factors and transcriptional activation of gliogenic factors (Rowitch 2004; Rowitch and Kriegstein 2010).

More recently, fibroblast growth factor (FGF), an extracellular signalling molecule, has been reported to be a key regulatory factor controlling the switch from neurons to astrocytes in the developing mice cortex. Moreover, the FGF signalling has been reported to be both essential and sufficient to change cell fates via a MEK/MAPK pathway (Duong et al. 2019). In addition, the CNTF-induced JAK/STAT (Bonni et al. 1997) and Notch/Sox9/NFIA signalling pathways, reported to regulate the fate switch, are also found to be stimulated by the FGF activation (Sloan and Barres 2014).

The responsiveness of the progenitor cells to these extracellular cues is the intrinsic property of these cells. CNTF-induced JAK/STAT pathway activation has been observed in early and mid-gestation progenitor as well as when these cells differentiate into neurons. The astrogenic fate acquisition of these cells is linked with demethylation of the astrocyte-specific genes. The DNAs in the promoters of the astrogenic genes, *GFAP*, *S100β*, *Aqp4* and *Clu*, are highly methylated in progenitors before late gestation, thus blocking their transcription and acquisition of astrogenic potential (Fan et al. 2005; Namihira et al. 2004, 2009). Generation of neurons and glia is also regulated by Notch pathway. Notch activation induces demethylation of the GFAP promoter including the STAT3 binding site by directly inducing the transcriptional activation of Notch-target genes, Nuclear Factor 1A (*Nf1a*), which act as a glial promoting factor. Notch-activated NFIA associate with the promoter of astrocyte-specific gene, *GFAP*, leading to the dissociation of DNMT1 (DNA methylating enzyme 1) from the promoter causing the demethylation of STAT3 binding site (Bajenaru et al. 2002; Garcia et al. 2004; Namihira et al. 2009). *Dnmt1* in early NPCs maintain the methylation of astroglial marker genes in early NPCS

enabling them to differentiate into neurons. Subsequently, the committed neuronal precursors and naïve neurons express notch ligands and activate notch signalling in nearby mid-late-gestational NPCs to promote their differentiation into the next lineage, i.e. astrocytes, by inducing the demethylation of the astrocytic genes (Namihira et al. 2009).

Demethylation of the GFAP promoter is crucial for astrocytic differentiation from neural stem cells (Yasui et al. 2017). NF1 binding sites have also been found in the promoters of other astrocyte-specific genes regulating their expression (Saadoun et al. 2005; Gopalan et al. 2006). Notch-activated NF1A in NPCs also potentiate cytokine-activated STAT1/3 binding with the demethylated promoters of the astrocytic-specific genes leading to the differentiation of astrocytes. NFIA helps to maintain the continued inhibition of neurogenesis by inducing Notch effector Hes5, further strengthening the role of NFIA in gliogenesis (Kang et al. 2012). Thus, NFIA is a key regulator of fate determination of NSCs and neuron-glia switch and is both necessary and sufficient for initiating prenatal glial fate specification as well as astrocytogenesis (Gronostajski 2000; Deneen et al. 2006; Namihira et al. 2009). Thus, NF1 genes (NF1a, NF1b and NF1x) are reported to play essential roles in astrogenesis, and deletion of either NF1A or NF1B has been reported to lead to a loss of GFAP expression in both the cortex (das Neves et al. 1999; Steele-Perkins et al. 2005) and spinal cord (Deneen et al. 2006), while misexpression of NF1A or NF1B was able to accelerate GFAP expression in astrocyte precursors, indicating their role in the terminal differentiation of astrocytes. Matuzelski and his group (2017) recently added to this information suggesting that NF1b-driven NF1x expression is necessary for the terminal differentiation of astroglial progenitors and maturation of astrocytes.

In the developing spinal cord, the astrocytes are generated mainly from the radial glia and the astrocyte progenitors of the early embryonic stages, which then undergo multiple rounds of cell divisions to generate the required number of astrocytes in the postnatal and adult spinal cord (Tien et al. 2012). However, in the developing mammalian cortex, the astrocytes are generated from four different sources in successive waves: (1) directly from radial glia (RG) and glia-restricted progenitors (GRPs) or glioblasts of the VZ of respective cortical regions, (2) from secondary progenitors of subventricular zones (SVZ), (3) through the local proliferation of the naïve astrocytes and (4) from NG2 glia of the postnatal and adult brain (Marshall et al. 2003; Noctor et al. 2004; Ge et al. 2012; Molofsky and Deneen 2015; Ge and Jia 2016; Naik et al. 2017). Radial glia are the main source of astrocytes in the early embryonic brain and generate GRPs and glioblasts first by asymmetric cell division. These GRPs, which are highly proliferative, are found between mid-late embryonic (E16–18) and early postnatal stages in mouse cortex (Burns et al. 2009; Magavi et al. 2012). These glial progenitors move radially from VZ/SVZ, undergoing proliferation while migrating and generating clusters of astrocytes in the cortical columns of the postnatal cortex (Levison et al. 1993; Luskin and McDermott 1994). Such clusters of astrocytes in the postnatal cortex suggest the local proliferation of GRPs and terminally differentiated astrocytes during migration and thereafter (Price and Thurlow 1988; Magavi et al. 2012). Finally, once the genesis ceases

from the VZ, the radial glia detach and move towards pial surface to transform into astrocytes. Once differentiated, the astrocytes derived from various sources have different proliferative potential once. Cortical appearance of differentiated astrocytes is detected first around E16 and peaks between postnatal (P) days 2 and 6, when the proliferation rate is highest generating more than 50% of the total astrocyte population by P28 (Qian et al. 2000; Ge et al. 2012; Naik et al. 2017). The rate of proliferation decreases subsequently even as the rest of the astrocytes are generated.

4 Morphological and Functional Maturation of Astrocytes

Astrocytes are the most predominant cell type in the brain accounting for approximately 80% of the total glia found in the central nervous system. For almost a century, the astrocytes have been categorised mainly into the protoplasmic and fibrous, especially based on their morphological appearance and location. Such features are attained during the late phase of astrocyte proliferation. The protoplasmic astrocytes populated in the grey matter develop small protoplasmic processes that enwrap the neural synapses as well as make contacts with the blood vessels. In contrast to this, the fibrous astrocytes of the white matter have elaborate processes that contact the myelinated axonal fibres and the nodes of Ranvier. In addition, both types of astrocytes express specific functional proteins, channels and receptors (Gallo and Deneen 2014; Haim and Rowitch 2017). In rodents, the astrocytic maturation takes place in the first few postnatal weeks. During this course of maturation, the astrocytes undergo a change in their morphology, connectivity and electrophysiological properties, and certain mature astrocyte markers, such as GFAP, S100β and Aqp4, are constantly upregulated (Bushong et al. 2004; Zhou et al. 2006). Various astrocyte-specific immunohistochemical markers, such as GFAP, GLAST and S100β, have been useful in the localisation and migratory pattern of astrocyte precursors as well as the sequence of astrocyte maturation (Takahashi et al. 1990; Yuasa 2001; Catalani et al. 2002).

However, the acquisition of complex morphology and the time course of astrocyte differentiation and maturation during prenatal and postnatal life in rats were revealed by experiments involving intracellular dye filling in fixed hippocampal slices by Bushong and his group in 2004. These studies have established that by the end of the second postnatal week, i.e. postnatal day (P) 14, the astrocytes appeared smaller with about a dozen long processes originating from the cell body and ending with a meshwork of filopodia-like structures, extensively overlapping territories with neighbouring astrocytes. Later by P21, these filopodia-like processes are transformed into the fine distal processes, and the astrocytes now appear more ramified and bushy and acquire cytoskeletal complexity and distinct and clear territorial domains (Catalani et al. 2002; Akdemir et al. 2020). Finally, any processes extending into the territory of others are pruned to acquire specific area as a result of competition between astrocyte processes and establish clear boundaries.

Subsequently, the perisynaptic processes appear from the terminal ends of the distal branches close to the synapses that in turn start enwrapping the synapses, thus

forming the third partner of the tripartite synapse (Freeman 2010; Savtchouk and Volterra 2018). During this time, the astrocytes secrete factors, such as thrombospondins and Hevin, that induce the initiation of silent synapses (Christopherson et al. 2005; Kucukdereli et al. 2011) followed by glypicans 4 and 6 that turn them into active contacts (Allen et al. 2012). Such a close relationship of the maturing astrocytes with the synapses suggests that both the neurons and the neural activity play a crucial role in their maturation (Stogsdill et al. 2017; Hasel et al. 2017). Moreover, the period of peak gliogenesis also corresponds with rapid blood vessel arborisation, extensions of neurites and initiation of synaptogenesis, which also suggests a close association between glial, vascular and synaptic components and may likely help in the establishment of glial-neuronal and glial-vascular interactions (Wise and Jones 1976; Bautch and James 2009). Astrocyte morphogenesis and synaptic plasticity are also regulated by neural activity through short-range signalling mechanisms, viz. neuroligin-neurexin (Stogsdill et al. 2017), Notch signalling (Hasel et al. 2017) and Epha4/ephrin-A3 (Filosa et al. 2009). This suggests that astrocyte maturation and functional synaptogenesis occur through interdependent mechanisms that are established during the critical period of neural development.

As the astrocytes mature, they start expressing specific functional genes for channels and receptors (GLT1, Cx43, Cx30, Kir4.1 and Aqp4), cytosolic proteins (GFAP, S100b, AldoC, GS) and secretary proteins (Thbs1, Gpc4,6, Hevin and SPARC) that provide them the characteristic functional ability during postnatal life (Freeman 2010; Akdemir et al. 2020). Finally, the mature astrocytes acquire morphologically complex architecture with highly ramified spongiform processes associated with the synapses (Witcher et al. 2007; Freeman 2010; Medvedev et al. 2014), competent to perform a diverse array of functions, viz. maintenance of homeostasis; providing metabolic and trophic support to the neurons; promoting synaptogenesis, neurotransmitter uptake and recycling; blood-brain barrier formation; development and functioning of synapses; modulation of synaptic contact and density of synapses; cognitive functioning; etc. (Allaman et al. 2011; Schiweck et al. 2018; Santello et al. 2019; Akdemir et al. 2020).

4.1 Astrocyte Markers

A variety of markers have been identified to track the astrocyte lineage along developmental timeline. The specified astrocyte precursors can be identified by three markers, glutamine aspartate transporter (GLAST), fatty acid binding protein/brain lipid binding protein (FABP7/BLBP) and fibroblast growth factor receptor 3 (FGFR3) (Shibata et al. 1997; Pringle et al. 2003; Anthony and Heintz 2007; Naik et al. 2017). GLAST is an early marker of gliogenesis induced by NF1A, and its expression corresponds with the gliogenic switch (Shibata et al. 1997; Deneen et al. 2006; Araque and Navarrete 2010). Both NF1A and NF1B have an intrinsic astrocyte bias and directly induce GLAST in astrocyte precursors marking their specification to generate astrocytes. The timing of induction of NF1A clearly

matches with the upregulation of GLAST in the VZ (Deneen et al. 2006). Moreover, the NF1 binding sites have been found in GFAP promoter inducing GFAP expression (Cebolla and Vallejo 2006). Subsequently, the astrocyte precursors migrate and proliferate and acquire terminal differentiation markers before they achieve morphological and functional maturation.

The most specified and commonly used astrocytic markers are GFAP and S100β that are used to identify immature astrocytes and to reveal their complete morphology along maturation (Catalani et al. 2002; Bushong et al. 2002, 2004; Donato et al. 2009; Allaman et al. 2011; Patro et al. 2015; Naik et al. 2017). Other markers reported to label astrocyte precursors and the mature astrocytes include aldolase C, Aldh1L1, BLBP, glutamine synthetase (GS), fatty acid binding protein FABP7, Aqp4, glutamine transporter 1 (GLT1), Sox9, connexins (Cx) 43 and 30 (Staugaitis et al. 2001; Liu et al. 2002; Furness et al. 2008; Molofsky et al. 2012; Szu and Binder 2016; Naik et al. 2017) and a novel astrocytic marker, NDRG2, for mature, nonreactive and non-proliferating astrocytes (Flugge et al. 2014; Zhang et al. 2019).

Keeping in mind the multifunctional roles of astrocytes in laying the functional architecture of the developing nervous system, any defect in the complex process of astrocytogenesis will certainly lead to profound abnormalities in the formation of the neural circuitry. A number of neurodevelopmental and psychiatric disorders are associated with the improper generation and dysfunctions of astrocytes during development, viz. Rett syndrome, fragile X syndrome, Down syndrome, Alexander's disease, autism, schizophrenia and others (Molofsky et al. 2012; Sloan and Barres 2014). These disorders are caused by either overproduction of astrocytes as seen in Down syndrome patients due to early gliogenic shift (Lu et al. 2011) or decrease in the number of astrocytes in the deeper cortical layers in schizophrenic patients. Altered astrocyte morphology along with altered expression of astrocyte markers, like GFAP, AQP4 and CX43, and abnormal synapse development leads to autistic pathology (Walsh et al. 2008). Precocious formation and maturation of astrocytes have also been implicated in RASopathies (Gauthier and Bukach 2007; Tidyman and Rauen 2009) and following early life stress such as maternal malnutrition (Naik et al. 2015, 2017) leading to neurocognitive delays and social and behavioural deficits. Thus, the neurodevelopmental defects leading to alterations in astrocytogenesis and functional neural circuits effectively contribute to mental retardation and increase the risk of developing adult-onset psychiatric diseases. The better understanding of the astrocyte development may shed new lights on these disorders and point to new curative therapies.

5 Genesis of Oligodendroglia

In the developing nervous system, various cell types are generated from NEPs in a strict hierarchical order: neurons are generated first, followed by astrocytes and then oligodendroglia in a predicted temporal order. Temporal differences in the intrinsic properties of the stem cells and their responsiveness to various growth factors are involved in the process. Temporal generation of separate cell population facilitates

the generation of neuronal population before the establishment of the glial system, as well as setting up of an appropriate balance between the number of neurons and glial cells (Burne et al. 1996; Calver et al. 1998; Barres and Raff 1999). The specification and differentiation of glia require signals from neurons, justifying their temporal order of generation in the developing nervous system. Oligodendrocytes are generated late in the development, as myelination is the last event to happen in neural development. Generation of these cells is required once most of the axons have been extended to their targets, so that the processes of oligodendrocytes initiate axo-glial contact, synthesise myelin and start enwrapping them. This also helps the oligodendrocytes to differentiate into various types, i.e. Type I–IV, depending on the location and diameter of the axons contacted. In rodents, most of the myelination occurs in the first two postnatal months of life, while in humans it is extended to the first two decades of life (Lebel et al. 2008; Mitew et al. 2013). However, there are evidences to point that in both humans and mice, the myelination continues throughout life, either to regenerate lost myelin or myelinating cells or to myelinate the axons which were earlier unmyelinated (Bartzokis et al. 2012; Young et al. 2013). Moreover, different parts of the CNS are myelinated at different developmental timelines, and most regions contain a mixture of both myelinated and unmyelinated axons. This requires strict genetic regulatory mechanisms to control the development of oligodendrocytes and the process of myelination of specific axon types.

6 Specification of Oligodendrocyte Precursors (OPCs)

Oligodendrocytes, the myelinating glia of the CNS, are generated from OPCs derived from the precursors in germinal zones of the developing CNS. At neuron-glia switch point, the stem cells stop generating neurons and produce progenitors with high proliferative potential that will generate glial cells in the presence of glia promoting signals. Notch signalling pathway and pro-glial transcription factors, Sox9 and NFI (discussed vide supra), are required for the transition to glial cell specification and generation. OPCs are specified as early as E10–12.5 in rodents, when the neurons are actively generated (Chandross et al. 1999; Zhou et al. 2000), while their differentiation into myelinating cells takes place during late gestation and postnatal life. In mouse spinal cord, the OPCs are generated in three succeeding waves: (1) around E12.5, from the ventral neuroepithelium within the pMN zone that also generates motor neurons (Lu et al. 2000), (2) from more dorsal domains around E15.5 (Fogarty et al. 2005) and (3) around birth from progenitor cells around the central canal or from NG2+ OPCs (Rowitch and Kriegstein 2010). Similarly, in the forebrain, the first wave of OPC production starts around E12.5 from medial ganglionic eminence and enteropeduncular area of the ventral telencephalon and accounts for ventrally derived OPCs (Spassky et al. 2001; Tekki-Kessaris et al. 2001), while the second and third waves from lateral and caudal ganglionic eminence at E15.5 and around birth from cortex account for dorsally derived OPCs (Kessaris et al. 2006). In the spinal cord, 80–90% of oligodendroglia are generated from ventrally derived OPCs, while in the forebrain majority of adult

oligodendroglia are derived from dorsally generated OPCs. Both the ventrally and dorsally generated OPCs show similarity in their electrophysiological properties (Tripathi et al. 2011) and can result in a compensatory expansion in opposing population following genetic ablation of either type to generate an appropriate population of oligodendrocytes and complement of myelin (Kessaris et al. 2006; Richardson et al. 2006). In addition, the OPCs are also generated from adult stem cells in the subventricular zone (SVZ) in the adult brain, and their specification may also be dependent on SHH and FGF2 (Azim et al. 2012; Ferent et al. 2013).

The fate specification of the neural precursor cells to the oligodendrocyte (OL) lineage is specifically controlled by the transcriptional factors regulating the dorsoventral patterning of the neural tube by gradients of sonic hedgehog (Shh), WNTs and bone morphogenetic (BMP) proteins. These extrinsic factors initially activate two transcriptional regulators of oligodendrocyte lineage, Olig1 and Olig2, in restricted domains of the ventral ventricular zone. Shh is secreted ventrally and is known to regulate the expression of various transcription factors, such as Nkx2.2 and Nkx6.1/6.2, Olig1 and Olig2 and Pax7, important for the development of OPCs in the forebrain and spinal cord (Tekki-Kessaris et al. 2001; Liu et al. 2003; Ortega et al. 2013; Rowitch and Kriegstein 2010). The effect of Shh is antagonised by dorsally expressed BMP and Wnt/β-catenin pathways (Mehler et al. 2000; Robertson et al. 2004; Ulloa and Marti 2010). Shh signalling is both necessary and sufficient for oligodendrocyte (OL) specification and production and works in a gradient to regulate the expression of transcription factors that pattern unique progenitors in the ventral neural tube (Briscoe and Novitch 2008; Dessaud et al. 2008). Both the concentration and temporal expression of Shh are important for establishing cell fate; the highest concentration induces the expression of Nkx2.2 in the most ventral progenitors, intermediate concentration of Shh induces intermediate neural tube fates (Olig2+ cells), and very low or no Shh induces the dorsal fates (Pax7+ cells) in the spinal cord.

Subsequent cross-regulatory interactions between the combinations of transcription factors within the progenitor domains establish sharp boundaries and regulate the identity and specification of cell types (Briscoe and Novitch 2008). Shh-induced expression of Olig2 is crucial for OPC specification, but the late stages of OPC maturation are Shh-independent (Orentas et al. 1999; Vallstedt et al. 2005). Once the OPCs are specified to the OL lineage, they migrate away from the source of Shh in the ventral midline to colonise the CNS. These committed OL precursors continue to express Olig1 and Olig2 as well as induce the expression of Nkx2.2 and Sox10 (Kuspert et al. 2011; Yu et al. 2013). Nkx2.2 plays an important role in the terminal differentiation of OPCs to OLs (Qi et al. 2001), while Sox9/10 proteins in OPCs induce the expression of PDGFRα that in turn promotes the survival and differentiation of OPCs by binding to its secreted ligand PDGF (Barres et al. 1993; Calver et al. 1998). The PDGFRα expression is a key event in OPCs' fate specification and their responsiveness to PDGF (Noble et al. 1988; Richardson et al. 1988) and represents all potentially myelinogenic cells (Sim et al. 2011). In addition, FGF2 also acts as a mitogenic factor either directly by promoting OPC proliferation or indirectly by promoting PDGFRα expression (Baron et al. 2000).

The differentiation of OPCs is tightly controlled both to regulate the timing of myelination during development and to maintain the OPCs' pool capable of proliferation and differentiation throughout life. The OPCs' pool is maintained by a number of transcription factors expressed by OPCs themselves, viz. Sox5, Sox6, Hes5, Id2 and Id4, that mediate an inhibitory influence acting on OPCs to prevent their differentiation and myelination (Emery and Lu 2015). Notch signalling pathway also helps to maintain the OPCs' pool and prevent their differentiation via the activation of Hes5 (Liu et al. 2006). In addition to OPCs, Sox5 and Sox6 are also expressed in neural progenitors and are downregulated during differentiation specifying their role in preventing differentiation (Stolt et al. 2006). In contrast, several other transcription factors induced early during lineage specification have a role in the differentiation of OPCs to OLs. These factors include Olig1, Olig2, Sox10, Nkx2.2, Myrf, Sip1, Nkx6.1/6.2, mash1, etc. (Li et al. 2009; Emery 2010). By virtue of the continued expression of Olig2 in the OL lineage, it has been designated as the oligodendrocyte lineage determination factor and controls both lineage specification, differentiation and myelination.

Extrinsic signals especially the neuronal activity and axonal influences have also been reported to play an important role in OPCs' proliferation, survival, differentiation and myelination (Emery 2010). Axonal contact, diameter of axons and axonally derived cell adhesion molecules and neurotrophins are variously described to regulate the myelination (Rosenberg et al. 2007; Mitew et al. 2013). Electrically active neurons signal to OPCs via the release of glutamate or ATP acting on their AMPA receptors or purinergic receptors (Bergles et al. 2000; Zonouzi et al. 2011). Other signalling molecules like FGFs, Neurotrophin-3, insulin-like growth factor 1 (IGF1), astrocyte-derived chemokines and neuregulin 1 (Nrg1) also control their proliferation and survival to ensure the generation of sufficient number of oligodendrocytes to produce myelin (Mitew et al. 2013).

Olig2 also plays an important role in neural subtype switch to specify the generation of motor neurons and oligodendrocytes in the pMN domain of the ventral spinal cord (Li et al. 2011; Zhu et al. 2012). The level of Oligs is crucial for the sequential control of fate determination and differentiation of motor neurons first and then glia (Lee et al. 2005). Initially, Ngn2 is co-expressed with Olig2 in a subset of progenitors in pMN, which act as the repressor of gliogenesis and thus promote motor neuron generation (Novitch et al. 2001; Zhou et al. 2002). Subsequently, Ngn2 is downregulated, and Nkx2.2 is expressed, defining the fate of OPCs in Olig1/2-expressing progenitors, suggesting that Ngn2 downregulation acts as a molecular neuron-glia fate switch (Rowitch and Kriegstein 2010). All glial progenitors express both NF1A and NF1B at the onset of gliogenesis throughout the developing spinal cord. NF1A is required for maintaining the expression of Olig2 in all OL lineage cells (Lu et al. 2002; Zhou and Anderson 2002). Subsequent to neuron-glia switch, NF1A/1B promotes astrocyte differentiation, and the NF1A function is suppressed in OPCs. This pro-astrocytic function of NF1A is antagonised by Olig2 in OPCs which co-express both, suggesting that gliogenic switch has an intrinsic astrocyte bias, which is then shifted to oligodendrocyte fate by Olig2 (Deneen et al. 2006).

7 Oligodendrocyte Differentiation

Oligodendrocyte differentiation is regulated by a myriad of both extrinsic and intrinsic factors, including extracellular signals, OPC-specific transcription factors, epigenetic modulators, miRNAs and intracellular signalling pathways (Eibaz and Popko 2019). Once the specification is established, the OPCs exit the cell cycle, become postmitotic and undergo a series of molecular changes that help them to differentiate into pre-myelinating/immature oligodendrocytes. Subsequently, these cells extend elaborate network of processes to contact nearby axons, wrap around them and finally switch on genes required for myelin synthesis and maintenance (Nave 2010). Differentiation of OPCs to OLs seems to be a terminal event, and there is no evidence of OLs undergoing in vivo dedifferentiation. A number of transcriptional regulators in OPCs/OLs are involved in coordinating these changes that require the expression of genes required to promote differentiation and repress other genes that prevent differentiation (Swiss et al. 2011; He and Lu 2013).

Chromatin remodelling is a key process that regulates the OL development by specific and coordinated gene expression via an activation of ATP-dependent SWI/SNF chromatin remodelling enzyme, Smarca-4/Brg1, that triggers the onset of differentiation (Emery 2010; Jacob et al. 2011). The activation of Brg1 is transcriptionally prepatterned by Olig2 and is both necessary and sufficient to trigger the onset of myelin-associated genes (Yu et al. 2013). Thus, Olig2 by promoting ATP-dependent chromatin remodelling makes the promoters of OL-specific genes accessible for transcription. Other transcription factors and regulatory molecules that collaborate to regulate OL differentiation include myelin gene regulatory factor (Myrf) and zinc finger protein 24 (Zfp24; earlier known as Zfp191). Myrf plays an important role in OL maturation and myelination and helps to maintain the OL identity in adult (Emery et al. 2009; Koenning et al. 2012) by directly binding to the targets of a number of genes involved in morphological development of OLs and myelination process, viz. cytoskeletal genes, lipid metabolism genes, transcriptions factors Smad7 and Nkx6 and myelin proteins, like myelin basic protein (MBP), proteolipid protein 1 (PLP1), cyclic nucleotide phosphodiesterase (CNP), myelin-associated glycoprotein (MAG) and myelin oligodendrocyte protein (MOG), all of which are essential for OL differentiation, myelination and paranodal formation (Cahoy et al. 2008; Emery et al. 2009; Bujalka et al. 2013). Expression of these genes is upregulated as a result of axonal contact and indicates the specified phase of OL differentiation. Zfp24 also influences the oligodendrocyte differentiation and myelination by binding to the regulatory regions of the required genes to control their expression (Elbaz et al. 2018). Continued expression of Olig2 and Sox10 is required for the maintenance of Myrf expression and its target genes involved in myelination (Goldman and Kuypers 2015). Thus, Olig2, Sox10, Nkx2.2, Zfp24 and Myrf act as the core regulatory network that regulates the OL differentiation and myelination. Olig2 directly activates Sox10, a factor considered to be the major determinant of oligodendrocyte differentiation. Both, in turn, maintain the expression of Olig2 in Sox10-positive cells relieving the repression on Nkx2.2. So, the expression of Olig2 and Nkx2.2 finally result in the initiation of OL differentiation

(Eibaz and Popko 2019). Olig1 synergistically interacts with Sox10 and promotes transcription of MBP in oligodendrocytes (Li et al. 2007). During maturation of OLs, the subcellular distribution of OLIG1 is shifted from nucleus to cytoplasm, which is required to promote membrane expansion, a vital requirement of myelin sheath formation (Niu et al. 2012). Nkx2.2 helps in maintaining the expression of MBP and PLP, while Nkx6.2 is required for the proper formation of the paranodes. At this transition to myelinogenesis, these immature oligodendrocytes start expressing surface lipid sulphatide and can be identified by O4 antibody (Sommer and Schachner 1981). With subsequent maturation, this lipid composition changes to galactocerebroside, and these cells become more mature and are recognised by O1 antibody (Bansal et al. 1989).

8 OPCs' Migration, Maturation and Myelination

After specification, OPCs migrate away from their point of origin to spread throughout the CNS to their final site of myelination, stop migrating, cease cell division, become stationary and terminally differentiate into postmitotic state, extend multiple processes and contact nearby axons. They remain highly proliferative while migrating to the cortex. Various extracellular signalling molecules regulate their migration and help them to travel great distances and also to ensure that OPCs are properly distributed throughout the CNS. These include the mitogenic factors, adhesion and contact molecules and chemokinetic cues. PDGFα and FGF2 are two mitogenic factors that power the OPCs' migration by independent signalling pathways (Baron et al. 2000; Miyamoto et al. 2008). During migration, the OPCs receive signals from ECM molecules and numerous cell adhesion molecules that work through adhesion or contact guidance, to achieve the homogeneous distribution of OPCs (Mitew et al. 2013). Across development, once OPCs are correctly positioned, they are transformed from simple bipolar morphology expressing PDGFRα, Olig1/2/Sox10 and NG2/CSPG4 to pre-myelinating immature oligodendroglia with more complex morphology bearing multiple processes and in addition acquire O4 and O1 antigens (Jiang and Nardelli 2016). These immature oligodendroglia are highly transient population in the mouse brain, but exist for at least 3 months in the human cerebral white matter, before their final maturation (Back et al. 2001, 2002). On further maturation, OLs become stationary and acquire complex morphology with perpendicular as well as long parallel processes following the axonal tracts and start expressing myelin synthesising proteins, MBP, PLP, MOG, MAG, etc. Finally, they transform into morphologically and electrophysiologically mature myelinating cells over time capable to myelinate the nearby axons.

Gradual developmental lineage progression of oligodendrocyte cells involves changes in their morphology, motility and a shift in expression of various cell surface proteins, used as stage-specific markers for oligodendrocyte maturation (Fig. 3). The acquisition of PDGFRα is well accepted as the most reliable marker of OPCs as it specifies the commitment of OPCs towards oligodendrocyte lineage. PDGFRα+ OPCs can be easily marked by E14 in rat brain with peak numbers during

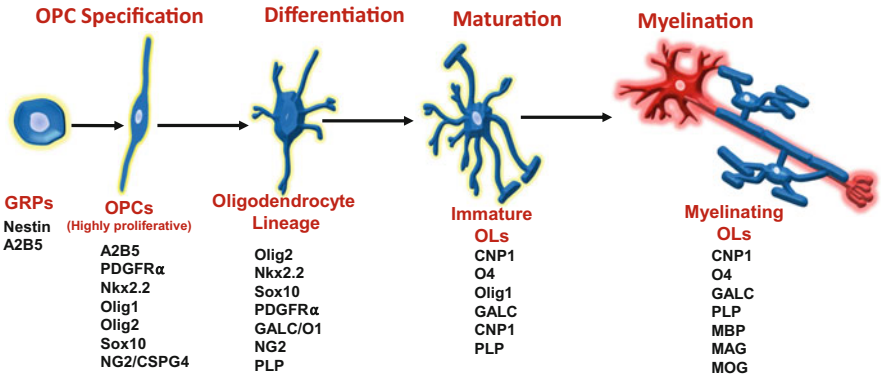

Fig. 3 Development of oligodendroglia: showing the temporal sequence of the specification, differentiation and maturation to myelinating oligodendrocytes with stage specific markers

the first 2 weeks of postnatal life, initially lying near the ventricles and later homogenously distributed in the cortical and subcortical areas (Patro et al. 2019). In addition, OPCs also express other marker proteins, viz. chondroitin sulphate proteoglycans (CSPG4) NG2, NKx2.2, Olig1, Olig2 and Sox10 (Zhang 2001; Mitew et al. 2013). With progressive maturation, the PDGFRα is downregulated, and various genes are expressed to mark the pre-myelinating oligodendrocytes (O1 and O4 antigens, CNP1 and PLP) and myelinogenic oligodendrocytes (CNP1, GALC/O1, PLP, MBP, MAG, MOG and MOBP) (Emery 2010; Mitew et al. 2013; Goldman and Kuypers 2015; Van Tilborg et al. 2017). PLP, an integral membrane protein, accounts for more than 50% of the total myelin protein in the CNS. It is expressed very early during development in oligodendrocyte lineage cells and plays a role in migration and differentiation in addition to its involvement in enhancing the stability and compaction of myelin.

Myelination occurs between postnatal (PND) days 10 and 14 and peaks around PND20 in rodents, while a significant amount of myelination in humans proceeds between 28 weeks of gestation and 3–5 years of age (Semple et al. 2013). Oligodendrocyte process extension, initial axonal contact and subsequent myelin thickening, guided by specific extracellular signalling molecules, all are highly instrumental for proper myelination required for rapid saltatory conduction and neurotransmission. Similar to neuronal apoptosis, apoptosis of glial cell population also takes place although the time course differs. While the peak neuronal apoptosis is recorded during prenatal life, excess oligodendrocytes undergo apoptosis a few days after the glial precursors undergo differentiation, i.e. the period of initial myelination when they contact axons, indicating that the axonally derived signals mediate the process to ensure that the surviving oligodendrocytes match the axonal surface area to be myelinated (McTigue and Tripathi 2008). Thus, proper axonal myelination and neuronal transmission rely on appropriate OPC specification, proliferation, migration and differentiation during development.

Myelin defects are seen in many human diseases, e.g. Pelizaeus-Merzbacher disease, a congenital leukodystrophy, where OPCs are unable to produce myelin because of mutation in PLP1 gene (Goldman et al. 2008). Oligodendrocytes are also a target in multiple sclerosis, which is an autoimmune disorder leading to myelin destruction. While OPCs extensively proliferate in demyelinating lesions, they fail to differentiate into mature OLs and effectively remyelinate, leading to disease progression (Franklin and Ffrench-Constant 2017). White matter injuries in the newborn and pre-term infants are another major reason for disability in survivors and may be a cause of cerebral palsy, epilepsy, cognitive delay and learning disabilities (Hack et al. 2002). Intra-generational protein malnutrition has also been reported to result in hypomyelination and demyelination lesions in corpus callosum bundle leading to behavioural deficits (Patro et al. 2019). Thus, any deleterious changes in oligodendrogenesis and myelination due to early life challenges or developmental disturbances may lead to neurodevelopmental disorders leading to persistent and long-term behavioural changes.

NG2 glia, the third major type of macroglia in the CNS, have gained attention in the last two decades and are known by multiple synonyms such as polydendrocytes, synantocytes, NG2 progenitor cells, OPCs or GluR cells (Nishiyama et al. 2009; Bergles et al. 2010; Kang et al. 2010; Trotter et al. 2010; Dimou and Gotz 2014; Dimou and Gallo 2015; Bender et al. 2020). The name NG2 defines the specific expression of nerve/glial antigen 2 which is an integral membrane chondroitin sulphate proteoglycan expressed on non-neuronal cells in the CNS. These NG2-expressing cells are found widely distributed in both white and grey matter of postnatal as well as adult CNS and make up to 5–10% of the total glia. NG2 glia are a distinct progenitor population in adult neurogenic niches, i.e. SVZ and hippocampal dentate gyrus, retain the capacity to proliferate and differentiate throughout life and constitute the major resident progenitor population, capable of responding to any type of injury signals and differentiating into multiple mature neural cell types to repopulate the lesion sites (Levine et al. 2001; Sellers et al. 2009; Richardson et al. 2011). However, their multipotential fate to give rise to neurons, astrocytes and oligodendrocytes is highly controversial, and the recent studies mostly emphasise that NG2 glia in both developing and adult brains are OPCs and, under normal physiological conditions, generate oligodendrocytes in the white matter and are the main source for remyelination of the naked axons in demyelinating disorders like multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and experimental autoimmune encephalitis (Magnus et al. 2008; Tripathi et al. 2010; Dimou and Gallo 2015). Majority of the grey matter NG2 glia do not differentiate into OLs and are maintained in their proliferative phenotype throughout life. These grey matter NG2 glia in rodent brain are known to make direct excitatory and inhibitory synaptic contacts with neurons in various brain regions (Bergles et al. 2010; Sakry et al. 2011). Neuronal activity is thought to regulate their fate and modulate their proliferation during development and regeneration.

Developmental origin of NG2 cells is similar to majority of other oligodendrocyte lineage cells from specific ventral and dorsal domains in both the spinal cord and forebrain (Nishiyama et al. 1996a; Tekki-Kessaris et al. 2001). NG2 cells first appear

after E15, about 2 days after the PDGFRα-expressive cells are seen in the CNS parenchyma. All these NG2-expressing cells co-express PDGFRα as well and are found scattered throughout the CNS parenchyma. These dual expressing cells outnumber the cells expressing PDGFRα only, and their number increases by the end of embryonic development reaching up to ~99%, except in the SVZ region where the cells express only PDGFRα (Nishiyama et al. 1996b; Tekki-Kessaris et al. 2001). SVZ is the major source for the generation of NG2 glia in both postnatal and adult CNS. This was shown by retroviral marking of perinatal SVZ where most of the NG2 cells in the developing neocortex and corpus callosum were generated from neonatal SVZ (Levison et al. 1993), while in the adult brain, the NG2 cells in the corpus callosum were generated from GFAP+ SVZ type B cells (Gonzalez-Perez et al. 2009). These NG2 cells in the SVZ are the transit-amplifying progenitors and do not express NG2. NG2 is expressed once they migrate towards the parenchyma surrounding SVZ (Aguirre et al. 2004; Cesetti et al. 2009; Komitova et al. 2009). NG2 glia are also generated through local proliferation to maintain their population in the adult CNS. These cells throughout their developmental stages bear stellate morphology with a central round soma and multiple long slender processes, thus deriving their name as polydendrocytes (Nishiyama et al. 2005, 2009). However, the number and length of the processes may change with age and their location in the CNS (Dawson et al. 2003). Similar to neurons, they express a variety of ligand and voltage-gated ion channels but are unable to generate action potential due to the relatively low density of voltage-gated sodium channels (Bergles et al. 2010; Larson et al. 2016).

NG2 glia have been broadly accepted to differentiate into mature myelinating oligodendrocytes (Zhu et al. 2008a; Kang et al. 2010; Dimou and Gallo 2015). However, there are temporal and spatial differences in the rate of generation of oligodendrocytes, i.e. faster in the white matter than in the grey matter. Moreover, their generation rate decreases with age prominently in the grey matter (Dimou et al. 2008; Kang et al. 2010; Zhu et al. 2011). In addition to oligodendrocytes, the NG2 glia have also been reported to generate interneurons of the hippocampus and olfactory bulb (Aguirre and Gallo 2004; Belachew et al. 2003), principal neurons in the piriform cortex (Rivers et al. 2008) and astrocytes in ventral areas of the forebrain and spinal cord (Zhu et al. 2008a, b; Guo et al. 2009). Although there is compelling evidence to accept that NG2 glia primarily give rise to oligodendrocytes, the findings from genomic studies indicate their lineage plasticity that is gradually lost with age.

Acknowledgements The Department of Biotechnology's support through the DBT-National Initiative on Glial Cell Research in Health and Disease is gratefully acknowledged. We are thankful to the Department of Biotechnology, the Department of Science and Technology and the Indian Council of Medical Research, Govt. of India, for their financial support through various grants. We are thankful to Dr. Urmishree Bedamatta for critically evaluating the manuscript for its English.

References

Aguirre A, Gallo V (2004) Postnatal neurogenesis and gliogenesis in the olfactory bulb from NG2-expressing progenitors of the subventricular zone. J Neurosci 24(46):10530–10541

Aguirre AA, Chittajallu R, Belachew S, Gallo V (2004) NG2-expressing cells in the subventricular zone are type C-like cells and contribute to interneuron generation in the postnatal hippocampus. J Cell Biol 165:575–589

Akdemir ES, Huang AY, Deneen B (2020) Astrocytogenesis: where, when, and how. F1000 Res 9: 233

Allaman I, Belanger M, Magistretti PJ (2011) Astrocyte-neuron metabolic relationship: for better and for worse. Trends Neurosci 34:76–87

Allen NJ, Bennett ML, Foo LC, Wang GX, Chakraborty C, Smith SJ, Barres BA (2012) Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. Nature 486:410–414. https://doi.org/10.1038/nature11059

Androutsellis-Theotokis A, Leker RR, Soldner F, Hoeppner DJ, Ravin R, Poser SW, Rueger MA, Bae SK, Kittappa R, McKay RD (2006) Notch signalling regulates stem cell numbers in vitro and in vivo. Nature 442:823–826

Anthony TE, Heintz N (2007) The folate metabolic enzyme ALDH1L1 is restricted to the midline of the early CNS, suggesting a role in human neural tube defects. J Comp Neurol 500:368–383

Araque A, Navarrete M (2010) Glial cells in neuronal network function. Philos Trans R Soc Lond B Biol Sci 365:2375–2381

Azim K, Raineteau O, Butt AM (2012) Intraventricular injection of FGF2 promotes generation of oligodendrocyte-lineage cells in the postnatal and adult forebrain. Glia 60:1977–1990

Babikian T, Prins ML, Cai Y, Barkhoudarian G, Hartonian I, Hovda DA, Giza CC (2010) Molecular and physiological responses to juvenile traumatic brain injury: focus on growth and metabolism. Dev Neurosci 32:431–441

Back SA, Luo NL, Borenstein NS, Levine JM, Volpe JJ, Kinney HC (2001) Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. J Neurosci 21:1302–1312

Back SA, Luo NL, Borenstein NS, Volpe JJ, Kinney HC (2002) Arrested oligodendrocyte lineage progression during human cerebral white matter development: dissociation between the timing of progenitor differentiation and myelinogenesis. J Neuropathol Exp Neurol 61:197–211

Bajenaru ML, Zhu Y, Hedrick NM, Donahoe J, Parada LF, Gutmann DH (2002) Astrocyte-specific inactivation of the neurofibromatosis 1 gene (NF1) is sufficient for astrocytoma formation. Mol Cell Biol 22(14):5100–5153

Bansal R, Warrington AE, Gard A, Ranscht B, Pfeiffer S (1989) Multiple and novel specificities of monoclonal antibodies O1, O4, and R-mAb used in the analysis of oligodendrocyte development. J Neurosci Res 24:548–557

Barnabé-Heider F, Wasylnka JA, Fernandes KJ, Porsche C, Sendtner M, Kaplan DR, Miller FD (2005) Evidence that embryonic neurons regulate the onset of cortical gliogenesis via cardiotrophin-1. Neuron 48(2):253–265

Baron W, Metz B, Bansal R, Hoekstra D, de Vries H (2000) PDGF and FGF-2 signaling in oligodendrocyte progenitor cells: regulation of proliferation and differentiation by multiple intracellular signaling pathways. Mol Cell Neurosci 15:314329

Barres BA, Raff MC (1999) Axonal control of oligodendrocyte development. J Cell Biol 147:1123–1128

Barres BA, Schmid R, Sendnter M, Raff MC (1993) Multiple extracellular signals are required for long-term oligodendrocyte survival. Development 118:283–295

Bartzokis G, Lu PH, Heydari P, Couvrette A, Lee GJ, Kalashyan G, Freeman F, Grinstead JW, Villablanca P, Finn JP, Mintz J (2012) Multimodal magnetic resonance imaging assessment of white matter aging trajectories over the lifespan of healthy individuals. Biol Psychiatry 72: 1026–1034

Bautch VL, James JM (2009) Neurovascular development: the beginning of a beautiful friendship. Cell Adh Migr 3:199–204

Bayer SA, Altman J, Russo RJ, Zhang X (1992) Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 14:83–144

Belachew S, Chittajallu R, Aguirre AA, Yuan X, Kirby M, Anderson S, Gallo V (2003) Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. J Cell Biol 161:169–186

Bender P, Jabs R, Steinhauser C (2020) Properties of human astrocytes and NG2 glia. Glia 68:756–767

Bergles DE, Jabs R, Steinhauser C (2010) Neuron-glia synapses in the brain. Brain Res Rev 63(1–2):130–137

Bergles DE, Roberts JD, Somogyi P, Jahr CE (2000) Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 405:187–191

Bonni A, Sun Y, Nadal-Vicens M, Bhatt A, Frank DA, Rozovsky I, Stahl N, Yancopoulos GD, Greenberg ME (1997) Regulation of gliogenesis in the central nervous system by the JAK/STAT signalling pathway. Science 278:477–483

Briscoe J, Novitch BG (2008) Regulatory pathways linking progenitor patterning, cell fates and neurogenesis in the ventral neural tube. Philos Trans R Soc B 363:57–70

Bronstein R, Kyle J, Abraham AB, Tsirka SE (2017) Neurogenic to gliogenic fate transition perturbed by loss of HMGB2. Front Mol Neurosci 10:153

Bujalka H, Koenning M, Jackson S, Perreau VM, Pope B, Hay CM, Mitew S, Hill AF, Lu QR, Wegner M, Srinivasan R (2013) MYRF is a membrane-associated transcription factor that autoproteolytically cleaves to directly activate myelin genes. PLoS Biol 11:e1001625

Burne JF, Staple JK, Raff MC (1996) Glial cells are increased proportionally in transgenic optic nerves with increased numbers of axons. J Neurosci 16:2064–2073

Burns KA, Murphy B, Danzer SC, Kuan CY (2009) Developmental and post-injury cortical gliogenesis: a genetic fate mapping study with nestin-CreER mice. Glia 57(10):1115–1129

Bushong EA, Martone ME, Ellisman MH (2004) Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. Int J Dev Neurosci 22:73–86. https://doi.org/10.1016/j.ijdevneu.2003.12.008

Bushong EA, Martone ME, Jones YZ, Ellisman MH (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J Neurosci 22:183–192

Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, Xing Y, Lubischer JL, Krieg PA, Krupenko SA, Thompson WJ (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci 28:264–278

Calver AR, Hall AC, Yu WP (1998) Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron 20:869–882

Catalani A, Sabbatini M, Consoli C, Cinque C, Tomassoni D, Azmitia E, Angelucci L, Amenta F (2002) Glial fibrillary acidic protein immunoreactive astrocytes in developing rat hippocampus. Mech Ageing Dev 123:481–490

Cebolla B, Vallejo M (2006) Nuclear factor-1 regulates glial fibrillary acidic protein gene expression in astrocytes differentiated from cortical precursor cells. J Neurochem 97:1057–1070

Cesetti T, Obernier K, Bengtson CP, Fila T, Mandl C, Holzl-Wenig G, Worner K, Eckstein V, Ciccolini F (2009) Analysis of stem cell lineage progression in the neonatal subventricular zone identifies EGFR+/ NG2− cells as transit-amplifying precursors. Stem Cells 27:1443–1454

Chandross KJ, Cohen RI, Paras P, Gravel M, Braun PE, Hudson LD (1999) Identification and characterization of early glial progenitors using a transgenic selection strategy. J Neurosci 19: 759–774

Chitnis A, Henrique D, Lewis J, Ish-Horowicz D, Kintner C (1995) Primary neurogenesis in Xenopus embryos regulated by a homologue of the drosophila neurogenic gene Delta. Nature 375:761-766.C

Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA (2005) Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120:421–433. https://doi.org/10.1016/j.cell.2004.12.020

das Neves L, Duchala CS, Godinho F, Haxhiu MA, Colmenares C, Macklin WB, Campbell CE, Butz KG, Gronostajski RM (1999) Disruption of the murine nuclear factor I-A gene (Nf1a) results in perinatal lethality, hydrocephalus, and agenesis of the corpus callosum. Proc Natl Acad Sci U S A 96(21):11946–11951

Dawson MRL, Polito A, Levine JM, Reynolds R (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 24(2):476–488

Deneen B, Ho R, Lukaszewicz A, Hochstim CJ, Gronostajski RM, Anderson DJ (2006) The transcription factor NFIA controls the onset of gliogenesis in the developing spinal cord. Neuron 52:953–968. https://doi.org/10.1016/j.neuron.2006.11.019

Dessaud E, McMahon AP, Briscoe J (2008) Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen regulated transcriptional network. Development 135(15):2489–2503

Dimou L, Gallo V (2015) NG2 glia and their functions in the central nervous system. Glia 63(8): 1429–1451

Dimou L, Gotz M (2014) Glial cells as progenitors and stem cells: new roles in the healthy and diseased brain. Physiol Rev 94(3):709–737

Dimou L, Simon C, Kirchhoff F, Takebayashi H, Gotz M (2008) Progeny of Olig2-expressing progenitors in the grey and white matter of the adult mouse cerebral cortex. J Neurosci 28: 10434–10442

Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, Giambanco I (2009) S100βs double life: intracellular regulator and extracellular signal. Biochim Biophys Acta 1793(6): 1008–1022

Duong TA, Hoshiba Y, Saito K, Kawasaki K, Ichikawa Y, Matsumoto N, Shinmyo Y, Kawasaki H (2019) FGF signalling directs the cell fate switch from neurons to astrocytes in the developing mouse cerebral cortex. J Neurosci 39(31):6081–6094

Eibaz B, Popko B (2019) Molecular control of oligodendrocyte development. Trends Neurosci 42(4):263–277

Elbaz B, Aaker JD, Isaac S, Kolarzyk A, Brugarolas P, Eden A, Popko B (2018) Phosphorylation state of ZFP24 controls oligodendrocyte differentiation. Cell Rep 23:2254–2263

Emery B (2010) Regulation of oligodendrocyte differentiation and myelination. Science 330:779–782

Emery B, Agalliu D, Cahoy JD, Watkins TA, Dugas JC, Mulinyawe SB, Ibrahim A, Ligon KL, Rowitch DH, Barres BA (2009) Myelin gene regulatory factor is a critical transcriptional regulator required for CNS myelination. Cell 138:172–185

Emery B, Lu QR (2015) Transcriptional and epigenetic regulation of oligodendrocyte development and myelination in the central nervous system. Cold Spring Harb Perspect Biol 7(9):a020461

Ernst M, Jenkins BJ (2004) Acquiring signalling specificity from the cytokine receptor gp130. Trends Genet 20:23–32

Fan G, Martinowich K, Chin MH, He F, Fouse SD, Hutnick L, Hattori D, Ge W, Shen Y, Wu H, ten Hoeve J (2005) DNA methylation controls the timing of astrogliogenesis through regulation of JAK/STAT signalling. Development 132:3345–3356

Ferent J, Zimmer C, Durbec P, Ruat M, Traiffort E (2013) Sonic hedgehog signaling is a positive oligodendrocyte regulator during demyelination. J Neurosci 33:1759–1772

Filosa A, Paixão S, Honsek SD, Carmona MA, Becker L, Feddersen B, Gaitanos L, Rudhard Y, Schoepfer R, Klopstock T, Kullander K (2009) Neuron-glia communication via EphA4/Ephrin-A3 modulates LTP through glial glutamate transport. Nat Neurosci 12(10):1285–1292

Flügge G, Araya-Callis C, Garea-Rodriguez E, Stadelmann-Nessler C, Fuchs E (2014) NDRG2 as a marker protein for brain astrocytes. Cell Tissue Res 357(1):31–41

Fogarty M, Richardson WD, Kessaris N (2005) A subset of oligodendrocytes generated from radial glia in the dorsal spinal cord. Development 132:1951–1959

Franklin RJM, Ffrench-Constant C (2017) Regenerating CNS myelin-from mechanism to experimental medicines. Nat Rev Neurosci 18:753–769
Freeman MR (2010) Specification and morphogenesis of astrocytes. Science 330(6005):774–778
Furness DN, Dehnes Y, Akhtar AQ, Rossi DJ, Hamann M, Grutle NJ, Gundersen V, Holmseth S, Lehre KP, Ullensvang K, Wojewodzic M (2008) A quantitative assessment of glutamate uptake into hippocampal synaptic terminals and astrocytes: new insights into a neuronal role for excitatory amino acid transporter 2 (EAAT2). Neuroscience 157(1):80–94
Gallo V, Deneen B (2014) Glial development: the crossroads of regeneration and repair in the CNS. Neuron 83(2):283–308. https://doi.org/10.1016/j.neuron.2014.06.010
Garcia AD, Doan NB, Imura T, Bush TG, Sofroniew MV (2004) GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. Nat Neurosci 7(11): 1233–1241
Gauthier I, Bukach C (2007) Should we reject the expertise hypothesis? Cognition 103:322–330
Ge WP, Jia JM (2016) Local production of astrocytes in the cerebral cortex. Neuroscience 323:3–9
Ge WP, Miyawaki A, Gage FH, Jan YN, Jan LY (2012) Local generation of glia is a major astrocyte source in postnatal cortex. Nature 484(7394):376–380
Goldman SA, Kuypers NJ (2015) How to make an oligodendrocyte. Developmemt 142:3983–3995
Goldman SA, Schanz S, Windrem MS (2008) Stem cell-based strategies for treating pediatric disorders of myelin. Hum Mol Genet 17:R76–R83
Gonzalez-Perez O, Romero-Rodriguez R, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A (2009) Epidermal growth factor induces the progeny of subventricular zone type B cells to migrate and differentiate into oligodendrocytes. Stem Cells 27:2032–2043
Gopalan SM, Wilczynska KM, Konik BS, Bryan L, Kordula T (2006) Nuclear factor-1-X regulates astrocyte specific expression of the alpha 1-antichymotrypsin and glial fibrillary acidic protein genes. J Biol Chem 281:13126–13133
Gronostajski RM (2000) Roles of the NFI/CTF gene family in transcription and development. Gene 249:31–45
Guo F, Ma J, McCauley E, Bannerman P, Pleasure D (2009) Early postnatal proteolipid promoter-expressing progenitors produce multilineage cells in vivo. J Neurosci 29:72567270
Hack M, Flannery DJ, Schluchter M, Cartar L, Borawski E, Klein N (2002) Outcomes in young adulthood for very-low-birth-weight infants. New Engl J Med 346:149–157
Haim LB, Rowitch DH (2017) Functional diversity of astrocytes in neural circuit regulation. Nat Rev Neurosci 18(1):31–41
Hasel P, Dando O, Jiwaji Z, Baxter P, Todd AC, Heron S, Márkus NM, McQueen J, Hampton DW, Torvell M, Tiwari SS (2017) Neurons and neuronal activity control gene expression in astrocytes to regulate their development and metabolism. Nat Commun 8(1):1–8
He F, Ge W, Martinowich K, Becker-Catania S, Coskun V, Zhu W, Wu H, Castro D, Guillemot F, Fan G, De Vellis J (2005) A positive autoregulatory loop of JAK/STAT signalling controls the onset of astrogliogenesis. Nat Neurosci 8:1025–1036
He L, Lu QR (2013) Coordinated control of oligodendrocyte development by extrinsic and intrinsic signaling cues. Neurosci Bull 29:129–143
Hirabayashi Y, Suzki N, Tsuboi M, Endo TA, Toyoda T, Shinga J, Koseki H, Vidal M, Gotoh Y (2009) Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. Neuron 63:600–613
Jacob C, Christen CN, Pereira JA, Somandin C, Baggiolini A, Lötscher P, Ozçelik M, Tricaud N, Meijer D, Yamaguchi T, Matthias P (2011) HDAC1 and HDAC2 control the transcriptional program of myelination and the survival of Schwann cells. Nat Neurosci 14:429–436
Jiang X, Nardelli J (2016) Cellular and molecular introduction to brain development. Neurobiol Dis 92:3–17
Kang P, Lee HK, Glasgow SM, Finley M, Donti T, Gaber ZB, Graham BH, Foster AE, Novitch BG, Gronostajski RM, Deneen B (2012) Sox9 and NFIA coordinate a transcriptional regulatory Cascade during the initiation of gliogenesis. Neuron 74(1):79–94

Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE (2010) Ng2+ cns glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron 68:668–681

Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD (2006) Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci 9:173–179

Koenning M, Jackson S, Hay CM, Faux C, Kilpatrick TJ, Willingham M, Emery B (2012) Myelin gene regulatory factor is required for maintenance of myelin and mature oligodendrocyte identity in the adult CNS. J Neurosci 32:12528–12542

Komitova M, Zhu X, Serwanski DR, Nishiyama A (2009) Ng2 cells are distinct from neurogenic cells in the postnatal mouse subventricular zone. J Comp Neurol 512:702–716

Kriegstein A, Alvarez-Byulla A (2009) The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32:149–184

Kucukdereli H, Allen NJ, Lee AT, Feng A, Ozlu MI, Conatser LM, Chakraborty C, Workman G, Weaver M, Sage EH, Barres BA (2011) Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC. Proc Natl Acad Sci U S A 108:E440–E449. https://doi.org/10.1073/pnas.1104977108

Kuspert M, Hammer A, Bosl MR, Wegner M (2011) Olig2 regulates Sox10 expression in oligodendrocyte precursors through an evolutionary conserved distal enhancer. Nucleic Acids Res 39:1280–1293

Larson VA, Zhang Y, Bergles DE (2016) Electrophysiological properties of NG2+ cells: matching physiological studies with gene expression profiles. Brain Res 1638:138–160

Lebel C, Walker L, Leemans A, Phillips L, Beaulieu C (2008) Microstructural maturation of the human brain from childhood to adulthood. Neuroimage 40:1044–1055

Lee SK, Lee B, Ruiz EC, Pfaff SL (2005) Olig2 and Ngn2 function in opposition to modulate gene expression in motor neuron progenitor cells. Genes Dev 19:282–294

Levine JM, Reynolds R, Fawcett JW (2001) The oligodendrocyte precursor cell in health and disease. Trends Neurosci 24:39–47

Levison SW, Chuang C, Abramson BJ, Goldman JE (1993) The migrational patterns and developmental fates of glial precursors in the rat subventricular zone are temporally regulated. Development 119(3):611–622

Li H, de Faria JP, Andrew P, Nitarska J, Richardson WD (2011) Phosphorylation regulates OLIG2 cofactor choice and the motor neuron-oligodendrocyte fate switch. Neuron 69:918–929

Li H, He Y, Richardson WD, Casaccia P (2009) Two-tier transcriptional control of oligodendrocyte differentiation. Curr Opin Neurobiol 19(5):479–485

Li H, Lu Y, Smith HK, Richardson WD (2007) Olig1 and Sox10 interact synergistically to drive myelin basic protein transcription in oligodendrocytes. J Neurosci 27:14375–14382

Liu A, Li J, Marin-Husstege M, Kageyama R, Fan Y, Gelinas C, Casaccia-Bonnefil P (2006) A molecular insight of Hes5-dependent inhibition of myelin gene expression: old partners and new players. EMBO J 25:4833–4842

Liu R, Cai J, Hu X, Tan M, Qi Y, German M, Rubenstein J, Sander M, Qiu M (2003) Region-specific and stage-dependent regulation of Olig gene expression and oligodendrogenesis by Nkx6.1 homeodomain transcription factor. Development 130:6221–6231

Liu Y, Wu Y, Lee JC, Xue H, Pevny LH, Kaprielian Z, Rao MS (2002) Oligodendrocyte and astrocyte development in rodents: an in situ and immunohistological analysis during embryonic development. Glia 40(1):25–43

Louvi A, Artavanis-Tsakonas S (2006) Notch signalling in vertebrate neural development. Nat Rev Neurosci 7:93–102

Lu J, Esposito G, Scuderi C, Steardo L, Delli-Bovi LC, Hecht JL, Dickinson BC, Chang CJ, Mori T, Sheen V (2011) S100B and APP promote a gliocentric shift and impaired neurogenesis in down syndrome neural progenitors. PLoS One 6:e22126

Lu QR, Sun T, Zhu Z, Ma N, Garcia M, Stiles CD, Rowitch DH (2002) Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. Cell 109: 75–86

Lu QR, Yuk DI, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH (2000) Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. Neuron 25:317–329

Luskin MB, McDermott K (1994) Divergent lineages for oligodendrocytes and astrocytes originating in the neonatal forebrain subventricular zone. Glia 11(3):211–226

Ma Q, Chen ZF, Barrantes IB, de la Pompa JL, Anderson DJ (1998) Neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. Neuron 20:469–482

Magavi S, Friedmann D, Banks G, Stolfi A, Lois C (2012) Coincident generation of pyramidal neurons and protoplasmic astrocytes in neocortical columns. J Neurosci 32(14):4762–4772

Magnus TI, Carmen J, Deleon J, Xue H, Pardo AC, Lepore AC, Mattson MP, Rao MS, Maragakis NJ (2008) Adult glial precursor proliferation in mutant SOD1G93A mice. Glia 56(2):200–208

Marshall CAG, Suzuki SO, Goldman JE (2003) Gliogenic and neurogenic progenitors of the subventricular zone: who are they, where did they come from, and where are they going? Glia 43:52–61

Matuzelski E, Bunt J, Harkins D, Lim JW, Gronostajski RM, Richards LJ, Harris L, Piper M (2017) Transcriptional regulation of Nfix by NFIB drives astrocytic maturation within the developing spinal cord. Dev Biol 432:286–297

McTigue DM, Tripathi RB (2008) The life, death and replacement of oligodendrocytes in the adult CNS. J Neurochem 107(1):1–19

Medvedev N, Popov V, Henneberger C, Kraev I, Rusakov DA, Stewart MG (2014) Glia selectively approach synapses on thin dendritic spines. Philos Trans R Soc Lond B Biol Sci 369(1654): 20140047

Mehler MF, Mabie PC, Zhu G, Gokhan S, Kessler JA (2000) Developmental changes in progenitor cell responsiveness to bone morphogenetic proteins differentially modulate progressive CNS lineage fate. Dev Neurosci 22:74–85

Mitew S, Hay CM, Peckham H, Xiao J, Koenning M, Emery B (2013) Mechanisms regulating the development of oligodendrocytes and central nervous system myelin. Neuroscience 276:29–47

Miyamoto Y, Yamauchi J, Tanoue A (2008) Cdk5 phosphorylation of WAVE2 regulates oligodendrocyte precursor cell migration through nonreceptor tyrosine kinase Fyn. J Neurosci 28:8326–8337

Molofsky AV, Deneen B (2015) Astrocyte development: a guide for the perplexed. Glia 63:1320–1329

Molofsky AV, Krenick R, Ullian E, Tsai HH, Deneen B, Richardson WD, Barres BA, Rowitch DH (2012) Astrocyte and disease: a neurodevelopmental perspective. Genes Dev 26:891–907

Morrison SJ, Shah NM, Anderson DJ (1997) Regulatory mechanisms in stem cell biology. Cell 88: 287–298

Naik A, Patro N, Seth P, Patro IK (2017) Intra-generational protein malnutrition impairs temporal astrogenesis in rat brain. Biology Open 6:931–942

Naik AA, Patro IK, Patro N (2015) Slow physical growth, delayed reflex ontogeny, and permanent behavioral as well as cognitive impairments in rats following intra-generational protein malnutrition. Front Neurosci 9:446

Nakashima K, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Kawabata M, Miyazono K, Taga T (1999) Synergistic signalling in fetal brain by STAT3-Smad1 complex bridged by p300. Science 284:479–482

Namihira M, Kohyama J, Semi K, Sanosaka T, Deneen B, Taga T, Nakashima K (2009) Committed neuronal precursors confer astrocytic potential on residual neural precursor cells. Dev Cell 16: 245–255

Namihira M, Nakashima K, Taga T (2004) Developmental stage dependent regulation of DNA methylation and chromatin modification in an immature astrocyte specific gene promoter. FEBS Lett 572:184–188

Nave KA (2010) Oligodendrocytes and the "micro brake" of progenitor cell proliferation. Neuron 65:577–579
Nishiyama A, Komitova M, Suzuki R, Zhu X (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat Rev Neurosci 10:9–22
Nishiyama A, Lin XH, Giese N, Heldin CH, Stallcup WB (1996a) Interaction between NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells is required for optimal response to PDGF. J Neurosci Res 43:315–330
Nishiyama A, Lin XH, Giese N, Heldin CH, Stallcup WB (1996b) Co-localization of Ng2 proteoglycan and pdgf alpha-receptor on O2a progenitor cells in the developing rat brain. J Neurosci Res 43:299–314
Nishiyama A, Yang Z, Butt A (2005) Astrocytes and NG2-glia: what's in a name? J Anat 207:687–693
Niu J, Mei F, Wang L, Liu S, Tian Y, Mo W, Li H, Lu QR, Xiao L (2012) Phosphorylated olig1 localizes to the cytosol of oligodendrocytes and promotes membrane expansion and maturation. Glia 60:1427–1436
Noble M, Murray K, Stroobant P, Waterfield MD, Riddle P (1988) Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. Nature 333:560–562
Noctor SC, Martínez-Cerdeño V, Ivic L, Kriegstein AR (2004) Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nat Neurosci 7(2):136–144
Novitch BG, Chen AI, Jessell TM (2001) Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron 31:773–789
Orentas DM, Hayes JE, Dyer KL, Miller RH (1999) Sonic hedgehog signaling is required during the appearance of spinal cord oligodendrocyte precursors. Development 126:2419–2429
Ortega JA, Radonjic NV, Zecevic N (2013) Sonic hedgehog promotes generation and maintenance of human forebrain Olig2 progenitors. Front Cell Neurosci 7:254
Patro N, Naik A, Patro I (2015) Differential temporal expression of S100β in developing rat brain. Front Cell Neurosci 9:87
Patro N, Naik AA, Patro IK (2019) Developmental changes in oligodendrocyte genesis, myelination, and associated behavioral dysfunction in a rat model of intra-generational protein malnutrition. Mol Neurobiol 56(1):595–610
Price J, Thurlow L (1988) Cell lineage in the rat cerebral cortex: a study using retroviral-mediated gene transfer. Development 104(3):473–482
Pringle NP, Yu WP, Howell M, Colvin JS, Ornitz DM, Richardson WD (2003) Fgfr3 expression by astrocytes and oligodendrocytes and their precursors: evidence that astrocytes and oligodendrocytes originate in distinct neuroepithelial domains. Development 130:93–102
Qi Y, Cai J, Wu Y, Wu R, Lee J, Fu H, Rao M, Sussel L, Rubenstein J, Qiu M (2001) Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor. Development 128:2723–2733
Qian X, Shen Q, Goderie SK, He W, Capela A, Davis AA, Temple S (2000) Timing of CNS cell generation: a programmed sequence of neuron and glial cell production from isolated murine cortical stem cell. Neuron 28:69–80
Rice D, Barone S Jr (2000) Critical periods of vulnerability for the nervous system: evidence from humans and animal models. Environ Health Perspect 108:511–533
Richardson WD, Kessaris N, Pringle N (2006) Oligodendrocyte wars. Nat Rev Neurosci 7:11–18
Richardson WD, Pringle N, Mosley MJ, Westermark B, Dubois-Dalcq M (1988) A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell 53:309–319
Richardson WD, Young KM, Tripathi RB, McKenzie I (2011) Ng2-glia as multipotent neural stem cells: fact or fantasy? Neuron 70:661–673
Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD (2008) Pdgfra/ng2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci 11:1392–1401

Robertson CP, Braun MM, Roelink H (2004) Sonic hedgehog patterning in chick neural plate is antagonized by a Wnt3-like signal. Dev Dyn 229:510–519
Rosenberg SS, Powell BL, Chan JR (2007) Receiving mixed signals: uncoupling oligodendrocyte differentiation and myelination. Cell Mol Life Sci 64:3059–3068
Rowitch DH (2004) Glial specification in the vertebrate neural tube. Nat Rev Neurosci 5:409–419
Rowitch DH, Kriegstein AR (2010) Developmental genetics of vertebrate glial-cell specification. Nature 468:214–222
Saadoun S, Papadopoulos MC, Watanabe H, Yan D, Manley GT, Verkman AS (2005) Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. J Cell Sci 118:5691–5698
Sakry D, Karram K, Trotter J (2011) Synapses between NG2 glia and neurons. J Anat 219(1):2–7
Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai HH, Wong M, Gupta N, Berger MS, Huang E, Garcia-Verdugo JM, Rowitch DH (2011) Corridors of migrating neurons in the human brain and their decline during infancy. Nature 478:382–386
Santello M, Toni N, Volterra A (2019) Astrocyte function from information processing to cognition and cognitive impairment. Nat Neurosci 22:154–166
Savtchouk I, Volterra A (2018) Gliotransmission: beyond black and white. J Neurosci 38(1):14–25
Schiweck J, Eickholt BJ, Murk K (2018) Important shapeshifter: mechanisms allowing astrocytes to respond to the changing nervous system during development, injury and disease. Front Cell Neurosci 12:261
Sellers DL, Maris DO, Horner PJ (2009) Postinjury niches induce temporal shifts in progenitor fates to direct lesion repair after spinal cord injury. J Neurosci 29:6722–6733
Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ (2013) Brain development in rodents and humans: identifying benchmarks or maturation and vulnerability to injury across species. Prog Neurobiol 106-107:1–16
Shibata T, Yamada K, Watanabe M, Ikenaka K, Wada K, Tanaka K, Inoue Y (1997) Glutamate transporter GLAST is expressed in the radial glia-astrocyte lineage of developing mouse spinal cord. J Neurosci 17:9212–9219
Sim FJ, McClain CR, Schanz SJ, Protack TL, Windrem MS, Goldman SA (2011) CD140a identifies a population of highly myelinogenic, migration-competent and efficiently engrafting human oligodendrocyte progenitor cells. Nat Biotechnol 29:934–941
Sloan SA, Barres BA (2014) Mechanisms of astrocyte development and their contributions to neurodevelopmental disorders. Curr Opin Neurobiol 27:75–81
Sommer I, Schachner M (1981) Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. Dev Biol 83:311–327
Spassky N, Heydon K, Mangatal A, Jankovski A, Olivier C, Queraud-Lesaux F, Goujet-Zalc C, Thomas JL, Zalc B (2001) Sonic hedgehog-dependent emergence of oligodendrocytes in the telencephalon: evidence for a source of oligodendrocytes in the olfactory bulb that is independent of PDGFR alpha signalling. Development 128:4993–5004
Staugaitis SM, Zerlin M, Hawkes R, Levine JM, Goldman JE (2001) Aldolase C/Zebrin II expression in the neonatal rat forebrain reveals cellular heterogeneity within the subventricular zone and early astrocyte differentiation. J Neurosci 21(16):6195–6205
Steele-Perkins G, Plachez C, Butz KG, Yang G, Bachurski CJ, Kinsman SL, Litwack ED, Richards LJ, Gronostajski RM (2005) The transcription factor gene *Nf1b* is essential for both lung maturation and brain development. Mol Cell Biol 25:685–698
Stiles J (2017) Principles of brain development. Wiley Interdiscip Rev Cogn Sci 8:1–2
Stiles J, Jernigan TL (2010) The basics of brain development. Neuropsychol Rev 20:327–348
Stogsdill JA, Ramirez J, Liu D, Kim YH, Baldwin KT, Enustun E, Ejikeme T, Ji RR, Eroglu C (2017) Astrocytic neuroligins control astrocyte morphogenesis and synaptogenesis. Nature 551(7679):192–197
Stolt CC, Schlierf A, Lommes P, Hillgartner S, Werner T, Kosian T, Sock E, Kessaris N, Richardson WD, Lefebvre V, Wegner M (2006) SoxD proteins influence multiple stages of oligodendrocyte development and modulate SoxE protein function. Dev Cell 11:697–709

Sun Y, Nadal-Vicens M, Misono S, Lin MZ, Zubiaga A, Hua X, Fan G, Greenberg ME (2001) Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms. Cell 104:365–376

Swiss VA, Nguyen T, Dugas J, Ibrahim A, Barres B, Androulakis IP, Casaccia P (2011) Identification of a gene regulatory network necessary for the initiation of oligodendrocyte differentiation. PLoS One 6:e18088

Szu JI, Binder DK (2016) The role of astrocytic Aquaporin-4 in synaptic plasticity and learning and memory. Front Integr Neurosci 10:8

Takahashi T, Misson JP, Caviness VS Jr (1990) Glial process elongation and branching in the developing murine neocortex: a qualitative and quantitative immunohistochemical analysis. J Comp Neurol 302:15–28

Takouda BJ, Katada S, Nakashima K (2017) Emerging mechanisms underlying astrogenesis in the developing mammalian brain. Proc Jpn Acad Sci B93:386–398

Tekki-Kessaris N, Woodruff R, Hall AC, Gaffield W, Kimura S, Stiles CD, Rowitch DH, Richardson WD (2001) Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon. Development 128:2545–2554

Tidyman WE, Rauen KA (2009) The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. Curr Opin Genet Dev 19:230–236

Tien AC, Tsai HH, Molofsky AV, McMahon M, Foo LC, Kaul A, Dougherty JD, Heintz N, Gutmann DH, Barres BA, Rowitch DH (2012) Regulated temporal-spatial astrocyte precursor cell proliferation involves BRAF signalling in mammalian spinal cord. Development 139(14): 2477–2487

Tripathi RB, Clarke LE, Burzomato V, Kessaris N, Anderson PN, Attwell D, Richardson WD (2011) Dorsally and ventrally derived oligodendrocytes have similar electrical properties but myelinate preferred tracts. J Neurosci 31(18):6809–6819

Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD (2010) Ng2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. J Neurosci 30:16383–16390

Trotter J, Karram K, Nishiyama A (2010) NG2 cells: properties, progeny and origin. Brain Res Rev 63(1–2):72–82

Ulloa F, Marti E (2010) Wnt won the war: antagonistic role of Wnt over Shh controls dorso-ventral patterning of the vertebrate neural tube. Dev Dyn 239:69–76

Vallstedt A, Klos JM, Ericson J (2005) Multiple dorsoventral origins of oligodendrocyte generation in the spinal cord and hindbrain. Neuron 45:55–67

Van Tilborg E, de Theije CG, van Hal M, Wagenaar N, de Vries LS, Benders MJ, Rowitch DH, Nijboer CH (2017) Origin and dynamics of oligodendrocytes in the developing brain: implications for perinatal white matter injury. Glia 66:1–18

Walsh CA, Morrow EM, Rubenstein JLR (2008) Autism and brain development. Cell 135:396–400

Wise SP, Jones EG (1976) The organization and postnatal development of the commissural projection of the rat somatic sensory cortex. J Comp Neurol 168:313–343

Witcher MR, Kirov SA, Harris KM (2007) Plasticity of perisynaptic astroglia during synaptogenesis in the mature rat hippocampus. Glia 55:13–23

Yasui T, Uezono N, Nakashima H, Noguchi H, Matsuda T, Noda-Andoh T, Okano H, Nakashima K (2017) Hypoxia epigenetically confers astrocytic differentiation potential on human pluripotent cell-derived neural precursor cells. Stem Cell Rep 8(6):1743–1756

Young KM, Psachoulia K, Tripathi RB, Dunn SJ, Cossell L, Attwell D, Tohyama K, Richardson WD (2013) Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. Neuron 77(5):873–885

Yu Y, Chen Y, Kim B, Wang H, Zhao C, He X, Liu L, Liu W, Wu LM, Mao M, Chan JR (2013) Olig2 targets chromatin remodelers to enhancers to initiate oligodendrocyte differentiation. Cell 152(1–2):248–261

Yuasa S (2001) Development of astrocytes in the mouse hippocampus as tracked by tenascin-C gene expression. Arch Histol Cytol 64:149–158

Zhang S-C (2001) Defining glial cells during CNS development. Nat Rev Neurosci 2:840–843
Zhang Z, Ma Z, Zou W, Guo H, Liu M, Ma Y, Zhang L (2019) The appropriate marker for astrocytes: comparing distribution and expression of three astrocyte markers in different mouse cerebral regions. Biomed Res Int 2019:9605265
Zhou M, Schools GP, Kimelberg HK (2006) Development of GLAST(+) astrocytes and NG2(+) glia in rat hippocampus CA1: mature astrocytes are electrophysiologically passive. J Neurophysiol 95:134–143
Zhou Q, Anderson DJ (2002) The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. Cell 109:61–73
Zhou Q, Wang S, Anderson DJ (2000) Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. Neuron 25:331–343
Zhu X, Bergles DE, Nishiyama A (2008a) NG2 cells generate both oligodendrocytes and grey matter astrocytes. Development 135:145–157
Zhu X, Hill RA, Dietrich D, Komitova M, Suzuki R, Nishiyama A (2011) Age-dependent fate and lineage restriction of single ng2 cells. Development 138:745–753
Zhu X, Hill RA, Nishiyama A (2008b) Ng2 cells generate oligodendrocytes and grey matter astrocytes in the spinal cord. Neuron Glia Biol 4:19–26
Zhu X, Zuo H, Maher BJ, Serwanski DR, LoTurco JJ, Lu QR, Nishiyama A (2012) Olig2-dependent developmental fate switch of NG2 cells. Development 139:2299–2307
Zonouzi M, Renzi M, Farrant M, Cull-Candy SG (2011) Bidirectional plasticity of calcium-permeable AMPA receptors in oligodendrocyte lineage cells. Nat Neurosci 14:1430–1438

Origin and Development of Microglia

Nisha Patro and Ishan Patro

Abstract

Microglia, the resident immune cells of the central nervous system (CNS), originate through primitive hematopoiesis from early embryonic erythromyeloid progenitors (EMPs) found in the extra-embryonic yolk sac (YS). These primitive macrophages act as sole microglial progenitors which initially appear in the YS blood islands and subsequently migrate into the embryo and enter the neuroepithelium to colonize the brain rudiment, where they proliferate locally and spread spatially within the CNS to account for the only original pool of the myeloid cells under normal physiological conditions. A number of transcription factors, signaling molecules, and growth factors are involved in the lineage commitment of the myeloid cells and the fate determination and the acquisition of cellular identity of the microglia. Early embryonic microglia bearing amoeboid morphology and high proliferative potential gradually transform into the slowly dividing ramified microglia with more complex process arbors, express microglia homeostatic marker genes, and acquire phenotypic and functional maturity. Any deviation or perturbation in the microglial development leads to the generation of dysfunctional microglia or their impaired maturation leading to the loss of developmental functionality and susceptibility to develop neurodevelopmental disorders.

Keywords

Erythromyeloid progenitors · Microglia origin · Extra embryonic yolk sac · Differentiation and maturation of microglia

N. Patro (✉) · I. Patro
School of Studies in Neuroscience, Jiwaji University, Gwalior, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_7

1 Introduction

Microglia are the resident tissue macrophages of the central nervous system and play a crucial role in the maintenance of the CNS in the health and disease (Ransohoff and Perry 2009; Prinz and Priller 2014). Immune defense and maintenance of the CNS homeostasis are the two distinct functional characteristics of the microglia. Microglia continuously monitor their surrounding environment by highly ramified dynamic processes in order to respond to any disturbance (Davalos et al. 2005; Lehnardt 2010) and initiate a program to support neuroprotection and remodeling (Minghetti and Levi 1998). Microglia are also key players in maintaining brain homeostasis under physiological conditions by controlling neuronal proliferation and differentiation and participating in modification and elimination of synaptic structures through active interactions with synaptic connections (Perry et al. 2010; Hughes 2012; Blank and Prinz 2013; Bialas and Stevens 2013).

The development of an organism is a highly orchestrated process, containing several critical windows during which dynamic and irreversible fate decisions are spatially and temporally coordinated. The brain development is a prolonged process that begins early embryologically and continues to mature even after puberty in terms of sharpening and refinement of synaptic connections (Van Ryzin et al. 2018). Microglia are functionally associated with such synaptic modifications and are crucial for activity-dependent refinement of the visual system during the critical developmental period (Stevens et al. 2007; Schafer et al. 2012). In the developing brain, microglia help in regulating the generation of appropriate number of neurons by supporting the proliferation, survival, and apoptosis of progenitors (Ueno et al. 2013; Shklover et al. 2015; Mosser et al. 2017). Moreover, the early wiring of the CNS, synaptic refinement and pruning, synapse formation and their functional maturation, and the overall buildup of synaptic network all depend on the intricate ability of microglia.

Microglia were first described as a class of resident CNS cells by del Río Hortega (1919) based on their typical morphological appearance bearing a tiny cell body and highly branched processes. Recently, Umpierre and Wu (2020) reviewed 100 years of microglia research focusing three major advances: (a) their transcriptional diversity focusing the regional species-specific, disease-specific, and developmental heterogeneity, (b) stem cell-based approaches to study human microglia, and (c) their remarkable adaptation and plasticity owing to their multifunctional roles in neural development, maintaining brain homeostasis and neurological disorders. However, to elucidate the precise physiological roles and functions of microglia in a healthy adult CNS, it is important to be aware of the in-depth knowledge of the transcriptional mechanisms, epigenetic factors, and developmental pathways involved in the origin of these cells.

2 Origin of Microglia

The ontogeny of microglia has been a subject of debate for long. The journey started with del Río Hortega (1919), who identified a small population of phagocytic motile cells in the CNS and proposed that mesodermal cells of the pia mater infiltrating the brain during early development might account for the presence of early microglial cells in the embryonic brain which subsequently migrate to various nerve centers. Moreover, most early investigators including del Río Hortega (1919) himself proposed the mesodermal origin of microglia from blood monocytes, based on their phenotypic similarities and the phagocytic property with mononuclear cells, as well as the concept that monocytes are recruited in the neonatal as well as adult brain during inflammatory conditions. Del Río Hortega, in 1939, further characterized these cells as the non-neuronal, non-astrocytic elements of the CNS and as distinct from oligodendroglia and termed them as the microglial cells. On the basis of these observations, scientists long believed that monocytes circulating in the blood were the progenitors of microglia, which finally replaced the ones that entered the brain through meninges during early development. These early conjectures about the origin of microglia led to vigorous debates inspiring scientists for more rigorous studies in this area. After intensive research for around 150 years, microglia have now been designated as a distinct and unique macrophage population residing in the CNS with a well-defined developmental origin.

The colonization of microglia in CNS parenchyma occurs much before the formation of neuroectodermally derived macroglia, i.e., astrocytes and oligodendroglia (Schlegelmilch et al. 2011; Swinnen et al. 2013). During the late twentieth century, two hypotheses were propagated regarding the origin of microglia: the first one supported the shared lineage with neurons and macroglia from neuroectoderm based on the in vitro studies (Fujita et al. 1981; Hao et al. 1991; Fedoroff et al. 1997), while the second hypothesis proposed that microglia are derived from the hematopoietic cells which give rise to all the tissue macrophages (Kaur et al. 1987; Ling 1994). However, more recent advances changed these notions and suggest that microglia and other macrophages of the CNS originate through primitive hematopoiesis from early embryonic erythromyeloid precursors (EMPs) found in the extra-embryonic yolk sac (Alliot et al. 1999; Schulz et al. 2012; Ginhoux et al. 2013; Kierdorf et al. 2013; Perdiguero et al. 2015; Hoeffel et al. 2015; Goldmann et al. 2016; Ginhoux and Prinz 2018). The primitive hematopoiesis begins in the yolk sac (YS) around embryonic (E) day 7.5 in mice and contributes to the generation of erythrocytes and tissue resident macrophages (Palis et al. 1999; Bertrand et al. 2005). The primitive hematopoiesis decreases with progressive embryogenesis and is gradually replaced by definitive hematopoiesis which takes place in aorta-gonad-mesonephros (AGM) of the embryo around E10.5. These progenitors derived from AGM act as hematopoietic stem cells (HSCs) or bone marrow cells (BM) and produce the entire hematopoietic cell population after E11.5 or birth, respectively (Bertrand et al. 2005; Cumano and Godin 2007).

The primitive macrophages initially appear in the blood islands of the YS and later migrate in the embryo through blood circulation after the circulatory system is

established by de novo formation and remodeling of blood vessels around E8–E10 (Walls et al. 2008; Ginhoux et al. 2010). The newly generated EMPs are characterized as $CD31^{+}$ and $C\text{-}Kit^{+}$ and develop via the macrophage ancestor population A1 ($CD45^{+}$, $CX3CR1^{low}$, $F4/80^{low}$) to A2 ($CD45^{+}$, $CX3CR1^{hi}$, $F4/80^{hi}$) committed microglial progenitor pool (Kierdorf et al. 2013; Schulz et al. 2012). These microglial progenitors enter the neuroepithelium by E9.5–E10, colonize the brain rudiment, and continue until the blood-brain barrier is established, around E13.5–14.5 (Alliot et al. 1999; Chan et al. 2007; Gomez-Nicola and Perry 2015; Kierdorf et al. 2013). Subsequently, they proliferate locally and spread spatially within the CNS to account for the only original pool of myeloid cells in the normal healthy brain (Hashimoto et al. 2013; Sheng et al. 2015). The BM-derived monocytes/macrophages from circulation infiltrate the brain only under inflammatory and pathological insults (Shechter et al. 2009, 2013). Thus, the CNS contain only YS-derived macrophages that account for total microglial population in the adult brain. In contrast, the definitive HSCs in the fetal liver (FL) and BM or maternal macrophages do not add to the CNS resident microglia pool. Moreover, the YS-derived fetal macrophages migrate into the brain much before the onset of the production of monocytes from the FL. These cells maintain high proliferative potential in both the YS and brain rudiment where they colonize (Naito et al. 1990, 1996; Takahashi and Naito 1993) and act as potential microglial progenitors initially in the YS and subsequently enter into the brain rudiment either through leptomeninges or from brain ventricles and proliferate extensively (Alliot et al. 1999; Swinnen et al. 2013). Several studies claim that apoptotic signals trigger the migration and spread of microglia in the embryonic brains of zebrafish (Casano et al. 2016; Xu et al. 2016) and rodents (Swinnen et al. 2013).

Mesodermal origin of microglia proposed by del Río Hortega was also strongly supported by several investigators based on their physiological and phenotypic similarities with macrophages (Murabe and Sano 1982,1983; Hume et al. 1983; Perry et al. 1985; Ginhoux and Prinz 2018). Microglia are now reported to bear phenotypic similarities with tissue macrophages and circulating monocytes, expressing macrophage-specific markers, viz., F4/80, Fc receptor, and CD11b, in both mouse (Perry et al. 1985) and humans (Akiyama and McGeer 1990). Ultrastructural evidences also established the shared features of microglia with phagocytes (Ling and Tan 1974; Ling 1981).

The origin of microglia from YS was confirmed after two decades of rigorous research involving all modern scientific tools. In a genetic fate mapping experiment by inducing Cre recombinase activity from two different loci, i.e., runt-related transcription factor 1 (Runx1) (Ginhoux et al. 2010) and colony-stimulating factor receptor 1 (CSF-1R; Schulz et al. 2012), via injection of tamoxifen into pregnant mice during E7.0–E8.5, the in vivo fate mapping of YS-derived cells was carried out. The investigators confirmed that the early YS progenitors are the major source of microglia based on their observations that large fraction of microglia in mice injected at E7.0–E8.0 were genetically labeled (Runx1+). There was a substantial decrease in the relative number of tagged microglia in mice injected from E8.0 onward, and it was almost absent in mice injected at E8.5, suggesting that the

microglia are derived only from the EMPs that appear in the YS around E7.5. Although Runx1 is expressed by both YS and FL hematopoietic progenitors, at E7.5, only the YS progenitors are $Runx1^{+}$ that are specifically and irreversibly tagged. These EMPs are characterized as $CSF1R^{hi}$ and C-Myb^{-} (transcription factor myeloblastosis) EMPs that give rise to YS macrophages to colonize the embryonic brain rudiment to generate microglia (Hoeffel et al. 2015; Hoeffel and Ginhoux 2015; Ginhoux and Guilliams 2016). The transcription factor Myb is required only for the generation of HSCs but not microglia and thus is an important factor to differentiate between the primitive and definitive hematopoiesis (Schulz et al. 2012).

The YS origin of microglia was found to be conserved across many species, viz., zebrafish (Herbomel et al. 1999) and avians (Cuadros and Navascues 1998). However, spreading of YS progenitors in both zebrafish and avian embryos are independent of blood circulation; the progenitors invade the whole cephalic mesenchyme and then to the brain in zebrafish (Herbomel et al. 1999) and enter through the pial surface in birds (Kurz et al. 2001), while in mice the YS progenitors migrate through blood circulation, as was confirmed by the complete absence of microglia in $NCX^{-/-}$ embryos due to the lack of heartbeat and defective circulation (Koushik et al. 2001; Ginhoux et al. 2010, 2013). Moreover, both microglia and blood vessels seem to influence each other's development, as $PU.1^{-/-}$ mice that fail to develop the required number of microglia also display the underdeveloped vascular network (Rigato et al. 2011; Mosser et al. 2017). However, microglia do not solely depend on the vascular system for invasion in the brain neuroepithelium, and alternate routes for the entry of microglia from meninges and ventricles have been reported (Cuadros and Navascues 1998). Moreover, the BM-derived macrophages are also known to invade the brain through blood circulation during inflammatory conditions, but these cells neither integrate into the microglial network nor contribute to the microglial pool (Ajami et al. 2011; Ransohoff 2011).

In addition to parenchymal microglia, other resident non-parenchymal brain macrophages of perivascular space, meninges, and choroid plexus also share a common origin from EMPs dependent on master regulators, Runx1, PU.1, and IRF8 (Schulz et al. 2012; Goldmann et al. 2016; Lopez-Atalaya et al. 2018). The non-parenchymal macrophages of CNS constitute the first line of defense against cellular and pathogenic components and are maintained throughout life via local proliferation and self-renewal (Reu et al. 2017; Tay et al. 2017) with the exception of choroid plexus cells that are constantly replaced by BM-derived monocytes throughout adult life (Goldmann et al. 2016). The embryonic development of both parenchymal microglia and non-parenchymal macrophages is tightly regulated by a variety of transcription factors, epigenetic remodeling, and signaling pathways (Lopez-Atalaya et al. 2018). Although the ontogeny of non-parenchymal macrophages has not been fully explored, a complex process that involves the integration of both intrinsic and epigenetic factors and extracellular signaling molecules helps to shape their genomic buildup leading to the activation of cell-specific transcriptional profiles as well as fate decisions (Crotti and Ransohoff 2016; Prinz et al. 2017).

3 Early Specification and Differentiation of Microglia

3.1 Transcription Factors Required for Microglia Development and Homeostasis

The development of microglia and CNS macrophages from the primitive cells is an extremely complex process and requires temporal expression of specific factors that drive their journey in the developing CNS to give rise to resident ramified microglia. The early specification of the YS precursors and the development of microglia are dependent on the combinatorial action of a number of transcription factors involved in lineage commitment of the myeloid cells and the fate determination and maintenance of cellular identity of brain macrophages (Kierdorf et al. 2013; Nayak et al. 2014; Prinz and Priller 2014; Prinz et al. 2017; Heinz et al. 2015; Lopez-Atalaya et al. 2018).

Runx1 is a transcription factor involved in the development of all hematopoietic lineage cells (Samokhvalov et al. 2007) and has been reported as a critical factor to define that parenchymal brain macrophages originate from embryonic YS (Ginhoux et al. 2010; Zusso et al. 2012). In addition, Runx1 also regulates the proliferation and differentiation of microglial cells and the acquisition of the ramified morphology typical of adult microglia, suggesting its role in their maturation (Zusso et al. 2012). They also suggested its importance in the transition of the activated amoeboid microglia back into ramified states. Runx1 also interacts with other transcriptional elements to regulate the expression of several genes essentially involved in the lineage-specific development of multipotent progenitors. Physical interaction of Runx1 and CCAAT enhancer binding protein (C/EBP) is required for the expression of colony-stimulating factor receptor 1 (CSF1R) essentially involved in the development and homeostasis of myeloid cell in mice (Zhang et al. 1996).

During early development (before E8), expression of Runx1 is restricted to blood islands of the YS. Later the $Runx^+$ committed microglial progenitors migrate to CNS during early embryonic life before the development of the nervous system and vascularization and then differentiate into microglia. At this point, these naïve $Runx^+$ microglia bear amoeboid morphology and undergo extensive proliferation. The *Runx* expression is maintained in these cells even after they exit the cell cycle and is lost progressively with their phenotypic transformation from amoeboid to ramified, which occurs around postnatal day 10 in mice (Ginhoux et al. 2010).

Runx1 regulates the expression of several genes related to hematopoietic development. PU.1, a member of the Ets (E-twenty-six) family of transcription factors (Rosenbauer and Tenen 2007), is a key factor directly regulated by Runx1 during both embryonic and adult hematopoiesis (Huang et al. 2008). PU.1, an essential myeloid lineage-determining transcription factor, is dynamically expressed in various myeloid origin cells, like macrophages, neutrophils, mast cells, B cells, and microglia (Nayak et al. 2014). PU.1 expression is necessary for the early development of YS microglia precursors, as evident from the fact that in mice lacking PU.1, all the microglia and CNS macrophages are absent although the uncommitted C-Kit^+ EMP cells remain unaffected (Beers et al. 2006; Kierdorf et al. 2013; Goldman et al.

2016). Moreover, both resting and activated microglia in rodents and humans show constitutive expression of PU.1 (Walton et al. 2000; Smith et al. 2013). PU.1 deficiency thus interferes in the maturation of YS-derived myeloid progenitors because in the absence of PU.1, they fail to express mature myeloid differentiation markers such as CSF1R, CD11b, and CD64 (Olson et al. 1995).

In addition to Runx1 and PU.1, interferon regulatory factor 8 (IRF8) is another master transcription factor which is expressed in myeloid lineage cells and is required in the cell fate decision of murine myeloid cells (Holtschke et al. 1996; Ginhoux et al. 2010; Kierdorf et al. 2013). IRF8 is a constitutively expressed nuclear factor in microglia, and its expression is crucial for the phenotypic determination of these cells (Minten et al. 2012). Microglial development is impaired when IRF8 is knocked out in mice (Kierdorf et al. 2013; Shiau et al. 2015). IRF8 is known to function both as a heterodimeric complex with PU.1 and independently as downstream target (Taniguchi et al. 2001; Kierdorf et al. 2013). Kierdorf and their group (2013) also demonstrated that experimental deletion of PU.1 in microglia leads to downregulation of IRF8. They also reported that both PU.1 and IRF8 are crucial for the proper development of microglia at A1 and A2 levels. PU.1 deletion leads to the complete absence of microglia, while IRF8 ablation results in an overall decrease in the density of microglia. Because PU.1, which is expressed exclusively in hematopoietic cells and is required for myeloid cell development, results in the reduction of the number of both A1 and A2 progenitor cells, the IRF deficiency leads to the reduction in A2 cell population, suggesting that both the factors act independently and IRF8 is specifically required for the generation of A2 cells (Kierdorf et al. 2013). More recently, the role of IRF8 in the maturation of microglia has been established with transcriptome-based profiling of YS-derived macrophages throughout the developmental phase and adulthood (Hagemeyer et al. 2016).

Apart from these three master transcription factors, other factors like Spalt-like transcription factors 1 and 3 (SALL1, SALL3), MEIS3 (Meis homeobox 3), and MAFB (MAF BZIP transcription factor B) are also involved in the development and function of microglia and maintenance of homeostasis of microglia during adult life (Mass et al. 2016; Matcovitch-Natan et al. 2016; Gosselin et al. 2017). SALL1 is expressed exclusively in microglia and helps in maintaining them in steady state by repressing a transcriptional program, as its deletion triggers their phenotypic transition to an inflammatory reactive state (Buttgereit et al. 2016).

More recently, small non-coding microRNAs (miRNAs) have also been found to participate in the development of microglia as regulatory factors. These miRNAs are expressed from early embryogenesis to adulthood and play an important role in gene expression (Gangaraju and Lin 2009). miR-124 is expressed specifically in CNS and is known to regulate adult neurogenesis and neuronal differentiation (Yu et al. 2008; Cheng et al. 2009). miR-124 is also highly expressed in resident microglia and plays a significant role in maintaining them in quiescent state by directly inhibiting the transcription factors C/EBPα and PU.1, thus preventing them from acquiring reactive phenotypes (Ponomarev et al. 2011). The deficiency of miRNAs directly switches the microglia to assume activated states (Varol et al. 2017).

3.2 Extrinsic Factors Required for Microglia Development and Homeostasis

Many signaling growth factors and intercellular interactions are also known to influence the differentiation, maturation, and fate decisions of these cells. Transforming growth factor β (TGFβ), colony-stimulating growth factor-1 (CSF-1), and interleukin-34 (IL-34) are known to influence the development of microglia, by promoting their survival as observed from in vitro studies (Butovsky et al. 2014; Bohlen et al. 2017). TGFβ signaling has been reported to induce the expression of molecular microglial signature genes and microRNA both in mice and in human that are typical of adult microglia and facilitates to distinguish them from other infiltrating macrophages in the CNS (Butovsky et al. 2014). It was further reported that withdrawal of TGFβ from culture medium led to the reduction in the number of microglia mainly due to the increase in apoptosis of these cells, signifying the role of TGFβ signaling in the survival and maintenance of microglia in vivo (Butovsky et al. 2014). TGFβ and its receptors are highly expressed in microglia (Butovsky et al. 2014) but at low levels in both neurons and glial cells (Flanders et al. 1991; Hamby et al. 2010). Mice deficient in TGFβ lack microglia, develop synaptic abnormalities and motor dysfunctions typical of neurodevelopmental disorders (Paolicelli et al. 2011; Butovsky et al. 2014), and may die by 3 weeks of age due to severe multifocal auto-inflammatory disorder (Shull et al. 1992).

Other signaling molecules critically required for microglial differentiation in both developing and adult mice are CSF-1, IL-34, and their receptor CSF-1R (Ginhoux et al. 2010; Nayak et al. 2014; Lopez-Atalaya et al. 2018; Prinz et al. 2017). CSF-1R is a growth factor receptor for hematopoietic cells and is expressed in YS macrophages and microglia at E9.5, is maintained throughout development, and is essentially required for the development and differentiation of EMPs into microglia. Natural null mutation to *Csf-1r* and *Csf-1* genes shows a dramatic reduction in the number of tissue macrophages and microglia (Dai et al. 2002; Ginhoux et al. 2010; Erblich et al. 2011). This suggests that CSF-1R signaling plays a key role in microglial homeostasis in mice in vivo (Pixley and Stanley 2004) by driving the differentiation of early YS progenitors into microglia (Metcalf 1985) and/or by providing a survival signal for the differentiating macrophages (Lagasse and Weissman 1997). Ginhoux and his group, in 2010, observed that microglial development is affected more by the absence of CSF-1R than its ligand CSF-1, leading to the identification of its second ligand, IL-34. IL-34, a neuron-derived cytokine highly expressed in postnatal mouse brain, was found to have a profound role in the development of microglia (Wang et al. 2012). IL-34 bind to CSF-1R even with higher affinity but at sites different than CSF-1 (Chihara et al. 2010) and are found to be highly conserved in mammalian and avian species than CSF-1. This suggests the significant role of CSF-1R signaling in homeostasis which was also confirmed in IL-34 knockout mice (Garceau et al. 2010; Greter et al. 2012; Wang et al. 2012). Moreover, the absence or blockade of CSF-1R in the YS macrophages impairs microglial differentiation in embryos (Ginhoux et al. 2010). In addition, DNAX activation protein 12 (DAP12), an adaptor protein for CSF-1R, and a triggering

receptor expressed on myeloid cells (TREM2) have also been reported to play a role in the genesis of microglia, as evident from the delay in the differentiation and migration of microglia in the developing CNS in DAP12- or TREM2-deficient mice (Nataf et al. 2005). In such conditions, these mice develop osteopetrosis due to lack of osteoclasts (Kaifu et al. 2003; Neumann and Takahashi 2007). Humans with mutation in DAP12 or TREM2 gene develop the Nasu-Hakola disease featured by bone cysts, fractures, and psychotic symptoms, leading to severe neurodegeneration and encephalopathy (Paloneva et al. 2000). In sum, all the above evidences clearly indicate the importance of various transcriptional regulators and growth factors in driving the differentiation and maturation of YS progenitors into the typical microglia of the developing CNS.

4 Colonization, Distribution, and Terminal Differentiation of Microglia in Developing Brain Parenchyma

In humans, the amoeboid microglia specified by the expression of Iba1, CD68, CD45, and MHCII enter the cortical plate by 4.5 weeks of gestation via pial surface and ventricles to seed the brain rudiment during early fetal life (Rezaie 2003; Rezaie et al. 2005; Monier et al. 2007) and then gather in clusters near or within developing white matter and proliferate extensively (Verney et al. 2010). In addition to vascularization, the timings of microglial colonization correspond well with the formation of radial glia, neuronal migration, and myelination. The microglia thus migrate along radial glia, axonal tracts, and vasculature to distribute into various CNS regions (Rezaie et al. 1999; Monier et al. 2007; Pont-Lezica et al. 2011; Mosser et al. 2017). As they reach to their final location, they start differentiating into ramified morphology, assume full ramified appearance by 35 weeks of gestation (Esiri et al. 1991; Kostovic and Judas 2002), and acquire their spatial territories with a very little overlap by the first few weeks after birth (Harry 2013). A similar pattern of colonization and distribution of microglia has been observed in mouse. Once they reach to their final location, they differentiate into process bearing ramified phenotypes. Only a limited number of proliferative and motile amoeboid microglial progenitors are first seen in the meninges and the lateral ventricles between E10 and E12, and they then migrate along tangential and radial pathways to colonize all CNS regions (Sorokin et al. 1992; Swinnen et al. 2013; Mosser et al. 2017). By GD15–16, F4/80^{+} cells appear in the brain parenchyma which then become ramified by GD18–19 and gradually become more ramified and differentiate completely (Perry et al. 1985; Kaur and Ling 1991). These cells maintain their proliferative potential and expression of CD34, CD45, and protein tyrosine phosphatase receptor type C (PTPRC) indicating their myeloid lineage throughout embryonic life (Davoust et al. 2006) or may even after birth, by the first postnatal week (Hristova et al. 2010). Recent reports by Askew and group (2017) suggested the infiltration of FL-derived monocytes in the brain peaking at P3, but these monocytes do not contribute to the adult microglial population as they soon undergo apoptosis. This suggests the intrinsic inability of the FL-derived monocytes to differentiate into mature ramified

microglia (Ginhoux et al. 2013). Subsequent fate mapping studies finally ruled out the contribution of monocytes in the adult microglial pool (Hoeffel et al. 2015; Sheng et al. 2015) and confirm that the adult microglia are derived from early YS progenitors only (Askew et al. 2017).

The microglial infiltration into the subventricular and ventricular zone of the cortex is facilitated by two main factors: (a) microglia NADH oxidase, Nox2, which generates superoxide ions and regulates CSF-1R/VEGFR-1 (vascular endothelial growth factor receptor-1)-mediated microglial chemotaxis (Lelli et al. 2013), and (b) production of chemokine CXCL12 (stromal cell-derived factor) by $Trb2^{+}$ intermediate neural progenitors, favoring the recruitment of microglia in the SVZ (Arno et al. 2014). The migration of microglia during embryogenesis is also regulated by matrix metalloproteinases (MMP-8 and MMP-9) secreted by microglia themselves that remodel the extracellular matrix and facilitate their migration and expansion (Kierdorf et al. 2013). Other factors reported to guide their migration include gradients of guidance cues, semaphorins and netrins (Spassky et al. 2002), chemoattractant molecules such as MCP1, MIP-1α (Rezaie et al. 2002), CXCL12 (Arno et al. 2014), or ligands of CSF-1R and VEGFR1 (Lelli et al. 2013). Finally, during postnatal life, microglia reach their final destinations in the CNS parenchyma by expressing CX3CR1 which interacts with neuronally derived chemoattractant, fractalkine CX3C1 (Hoshiko et al. 2012; Arnoux and Audinat 2015). In addition, several signals originating from neurons undergoing apoptosis/programmed cell death (PCD) during embryonic and postnatal development or axonal degeneration also attract microglia (Perry et al. 1985; Rakic and Zecevic 2000). This is supported by a number of investigations showing the close association of microglia with apoptotic neurons in various CNS regions, like the neocortex (Upender and Naegele 1999), cerebellum (Marin-Teva et al. 2004), retina (Moujahid et al. 1996), hippocampus (Wakselman et al. 2008), and spinal cord (Rakic and Zecevic 2000). However, there are a few reports that even oppose the dependence of microglia colonization in the CNS on neuronal apoptosis (Eyo et al. 2015).

During embryonic development, microglial progenitors possess high proliferative potential as observed in the spinal cord, corpus callosum, hippocampus, and retina (Dalmau et al. 2003; Alliot et al. 1991; Rigato et al. 2011, 2012). Active proliferation of these progenitors essentially contributes to the colonization of microglia in the brain parenchyma. The process of proliferation is tightly regulated by various signaling factors including granulocyte macrophage colony-stimulating factor (GM-CSF), CSF-1, neurotrophin-3 (NT3), interleukins 4 and 5 (IL-4, IL-5), etc. (Navascues et al. 2000). Cell death in the developing brain also triggers microglial proliferation by the upregulation of a mitogenic cytokines and macrophage migration-inhibiting factor (MIF; Arno et al. 2014). The proliferation of microglia is extended into early postnatal life (Arnoux et al. 2013) and then gradually decreases and becomes stable by the second to third weeks postnatally in rodents (Marin-Teva et al. 1999). Once stable, the population of microglia is maintained throughout postnatal and adult life by a finely tuned balance between proliferation and apoptosis (Askew et al. 2017).

In humans, the fully developed microglia are observed by 35 weeks of gestation (Esiri et al. 1991) and then spread throughout the brain to occupy defined spatial territories during the first 2 weeks after birth (Rezaie and Male 2003). This specifies the area of surveillance for each cell. In this process, the amoeboid microglia decrease in number, while the highly ramified microglia bearing long thin branched processes increase (Wu et al. 1994; Monier et al. 2006). On the contrary, such transition in rat and mice is initiated by GD18–19 with the appearance of ramified $F4/80^{+}$ cells in brain parenchyma. These cells become gradually more ramified to become fully differentiated during the first postnatal month (Perry et al. 1985; Kaur and Ling 1991; Orlowski et al. 2003). By PND15, the ramified microglia are seen throughout the brain parenchyma with clearly demarcated boundaries and minimal overlap (Perry et al. 1985).

During the late embryonic and first 2 weeks of postnatal development, periventricular white matter microglia highly express Runx1. During this phase, Runx1 is involved in the transition of microglia from activated amoeboid states to the deactivated ramified states (Zusso et al. 2012). Runx proteins are known to coordinate the cell cycle exit with the gradual progression to a more developmentally mature state in many cell lineages (Kagoshima et al. 2007; Appleford and Woollard 2009). Thus, Runx1 in postnatal brain represses the proliferative ability of amoeboid microglia via a mechanism that secures the transition from actively proliferating amoeboid state to a slowly dividing typical ramified microglia (Ajami et al. 2007). In addition, the signals derived from mature neurons are also reported to promote the differentiation of immature amoeboid microglia to process bearing mature microglia. This suggests that interactions of microglia with neurons and withdrawal of signals coming from apoptotic neurons may be related with their maturation (Kostovic and Judas 2002). Further research suggests that maturation of microglia clearly coincides with the maturation of neurons, astrocytes, and oligodendrocyte population, suggesting that all these cells depend on each other for their maturation (Harry and Kraft 2012).

By birth, only 30% of the early microglial progenitors with amoeboid morphology persist in the rodents which finally differentiate and contribute to the mature ramified microglial population (Wang et al. 2002). During PND5–15 (critical period of postnatal microglial development), microglia gradually accumulate and escalate their number drastically with an increased ratio of ramified/amoeboid. The ramified microglia bear more complex process arbors and cytoplasmic material. In the later part of the maturation window, i.e., by PND15, microglia begin to adopt a mature phenotype, become highly ramified, and get well distributed throughout the brain, enabling surveillance of the majority of parenchyma. These microglia have reduced proliferative ability, show more heterogeneity, and express specific set of cell surface markers typical of mature microglia (Harry and Kraft 2012). After a peak, the microglia numbers decrease to adult level in the third postnatal week (Nikodemova et al. 2015). By PND20, the steady-state microglial population is well established and remains stable till there is any stimulation or disturbance, with no evident proliferation or turnover from systemic population. The final stage of microglial maturation involves intense ramification of microglial processes which provides

microglia the ability to initiate synaptic pruning functions, essentially required for neural circuit formation (Paollicelli et al. 2011). Amoeboid microglia of the developing brain, however, suggest that they are in activated states, but they differ the activated adult microglia seen in inflammatory and neurodegenerative diseases in terms of their gene expression profile (Lenz and Nelson 2018).

Resting/ramified microglia in the mature CNS evenly tile the CNS parenchyma, participate in the formation of a complex cellular architecture, and constantly survey the local territories (Nimmerjahn et al. 2005). Such tiling pattern is largely established during the seeding of early YS-derived progenitors (Ginhoux et al. 2010). The long-standing view that homogenous population of microglia tile the entire brain and perform the same functions in all brain regions has been challenged with more recent data suggesting that microglia represent a population of complex and functionally diverse cells (Lopez-Atalaya et al. 2018). Such microglial heterogeneity develops during both postnatal life and adulthood as a result of signaling from local environmental cues that continuously instruct and shape the identity of microglia into region-specific phenotypes (De Biase et al. 2017). Constant interaction with these environmental factors is required to support their phenotypes, identity, and plasticity (Lopez-Atalaya et al. 2018) although the nature of these signals is largely unexplored.

Matcovitch-Natan and group in 2016 classified the microglial development into three transcriptional stages in addition to YS stage, i.e., early (E10.5–E14), pre- (E14–P9), and adult (4 weeks onward after birth) microglia, by doing RNA sequencing to measure the gene expression of YS myeloid progenitors and microglial progenitors and microglia in brains at various stages of embryonic and postnatal development as defined above. From these studies, they suggested that a number of genes are differentially expressed in microglia across developmental timeline reflecting their stage-related activities in the brain. Moreover, the temporal expression profile of microglial development consists of two major transitions, from i) early microglia to pre-microglia around E13.5–E14.5 and ii) pre-microglia to adult microglia, a few weeks after birth. These transitions are controlled by coordinated transcriptional events and are susceptible to even subtle genetic or environmental perturbations which can disrupt the stage-specific functions of developing/maturing microglia causing disturbed brain homeostasis and may even lead to neurodevelopmental abnormalities (Matcovitch-Natan et al. 2016).

Under normal physiological conditions, expression of microglia-enriched genes sets the maturation of microglia. These genes are also known as microglial homeostatic markers in the mature microglia (Beutner et al. 2013; Butovsky et al. 2018; Masuda et al. 2020a, b) and include *Tmem119*, *Olfm13*, *P2yr12*, *Sall1*, *Hexb*, *Gpr34*, *Fcrls*, or *SiglecH*. These genes are upregulated during the first 2 weeks of postnatal life in mice and are essentially required for mediating microglial functions under normal physiological conditions (Butovsky and Weiner 2018; Spittau et al. 2020) and correspond well with the activation of TGFβ signaling, which is peaked at P7 in mice and precedes to upregulate the microglia-specific markers (Bennett et al. 2016; Attaai et al. 2018). TGFβ derived from neurons (TGFβ1) and NG2 glia (TGFβ2) is known to initiate the process of postnatal microglial maturation (Attaai et al. 2018;

Zoller et al. 2018; Liu and Aguzzi 2019). Thus, the current view is that TGFβ signaling is critically required for microglial development and maturation (Spittau et al. 2020; Wurm et al. 2021). The impairment in TGFβ signaling by deletion of its receptor causes loss of microglia maturation, and lack of expression of microglial homeostatic marker genes rather results in the expression of marker genes expressed in primed, aged, and immune-activated microglia (Holtman et al. 2015; Zöller et al. 2018). More so, the affected microglia exhibit the activated phenotypes, suggesting the role of TGFβ in the regulation of microglial activation (Zoller et al. 2018; Arnold et al. 2019). In animal models of Alzheimer's and Parkinson's diseases, TGFβ signaling was effective in combating neuroinflammation and promoting neuroprotection, further specifying its role in the regulation of microglial activation (Chen et al. 2015, 2017). In addition to neuronally derived TGFβ, IL33 derived from astrocytes and presence of CD4+ T cells have also been found to trigger postnatal microglial maturation (Vainchtein et al. 2018; Pasciuto et al. 2020).

In addition to TGFβ signaling, neurons and microglia also interact through other receptor-ligand interactions during microglial development, viz., CD200/CD200R (Lyons et al. 2007; Shrivastava et al. 2012) and CX3CL1/CX3CR1 (Cardona et al. 2006) signaling, that are required to maintain microglia in normal physiological quiescent state and prevent their activation.

5 Microglial Markers to Study Their Phenotype, Distribution, and Functions

Microglial markers include proteins that are either intracellular or secreted or expressed on their surface. These marker proteins usually help us to discriminate them from peripheral macrophages. The general microglial markers are transmembrane protein 119 (TMEM119), purinergic receptor P2Y12R (Butovsky et al. 2014), ionized calcium binding adaptor molecule 1 (Iba 1; Yun et al. 2018), glycoprotein F4/80 (Lin et al. 2005), complement receptor 3 (CR3/Cd11b; Jeong et al. 2013), cluster of differentiation (CD) receptors (Cd14, CD45, CD68, CD80, and CD115), HLA-DR, a MHC class II cell surface receptor (Zanoni et al. 2011; Fadini et al. 2013; Jenkins et al. 2013; Rice et al. 2017; Jurga et al. 2020), fractalkine receptor CX3CR1 (Jones et al. 2010), ferritin (Holland et al. 2018), high affinity immunoglobulin epsilon receptor subunit gamma (FCER1G), and vimentin (Mukherjee et al. 2016). Most of these markers are common to both resting and activated microglia, and the activated microglia can be distinguished by the upregulated expression of these marker proteins. In steady-state microglia, CD45 levels are much lower than the perivascular macrophages, and thus it appears to phenotypically distinguish the two populations (Greter and Merad 2012). The microglial phenotyping has revealed a great heterogeneity in terms of (a) ratio/density in different regions of brain with highest in the forebrain and lowest in the cerebellum and (b) their cellular morphology, compact with small round cell soma and short thick processes/elongated cell body with long processes/radially branched cells (Lawson et al. 1990; Das Sarma et al. 2013). In addition, the microglia also differ on the basis of the set of protein

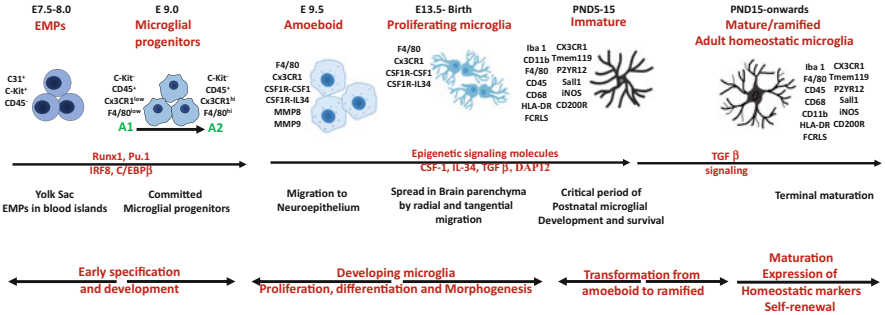

Fig. 1 Schematic diagram showing the development of microglia in the central nervous system from the YS progenitors along with the transcription factors, signaling molecules and growth factors involved in lineage commitment of the myeloid cells, and the fate determination and the acquisition of cellular identity of the mature ramified microglia

markers expressed in different brain regions (Bottcher et al. 2019; Masuda et al. 2020a, b). Greater spatial and temporal microglial diversity has been reported in the human brain than in rodents (Bottcher et al. 2019; Masuda et al. 2019). The human and rodent microglia also differ in some of their marker proteins (Jurga et al. 2020). The development of microglia from the YS progenitors, guided by a number of transcription factors, signaling molecules, and growth factors involved in lineage commitment of the myeloid cells, and the fate determination and the acquisition of cellular identity of the microglia have been summarized in Fig. 1.

6 Perturbations in Microglial Development and Consequences

Microglia are the active players in shaping the developing nervous system by playing active roles in pruning and modifiability of synaptic connections, regulation of neural circuitry formation, neuronal survival, myelination, stability and maintenance of synaptic connections, etc. (Paolicelli et al. 2011; Ueno et al. 2013; Hagemeyer et al. 2017; Wang et al. 2016). Thus, any perturbation in the development and maturation of microglia will have deleterious effects on the development of CNS during embryonic and postnatal life.

TGFβ signaling is essential for microglial maturation during critical postnatal window, as described (vide supra). Defects or depletion of TGFβ signaling has been reported to severely affect the postnatal myelination of gray and white matter tracts by interfering with the maturation of oligodendroglia and result in interneuron loss and severe neuromotor dysfunctions and spasticity, typical of many human neurodevelopmental disorders. Such defects are mediated by arrest of microglial maturation and persistent activation of dysmature microglia in the absence of TGFβ signaling (Arnold et al. 2014, 2019).

Mutations in a number of microglia-related genes are known to affect early brain development and cause diseases named as microgliopathies associated with neuropsychiatric and neurological disorders (Rademakers et al. 2012). The Nasu-Hakola disease (NHD) and hereditary diffuse leukoencephalopathy with spheroids (HDLS) are human microgliopathies resulting from mutations in genes expressed in developing microglia. NHD is a rare autosomal microgliopathy caused by a recessive mutation in DAP12 or triggering receptor TREM2 gene and is characterized by bone cysts, bone fractures, and psychotic symptoms, leading to severe neurodegeneration and encephalopathy (Paloneva et al. 2001; Bianchin et al. 2004). These genes in CNS are expressed by microglia and are required for their proper development and functioning as well as long-term preservation (Roumier et al. 2004; Wakselman et al. 2008; Otero et al. 2009). Genetic analysis of DAP12-deficient microglia indicates the downregulation of a number of genes involved in neurite formation (Pont-Lezica et al. 2014) and their ability to clear apoptotic neurons in culture and in developing mouse CNS (Takahashi et al. 2005; Wakselman et al. 2008). Thus, compromised TREM2-DAP12 signaling during prenatal life may lead to the generation of dysfunctional microglia causing defects in synaptic functions as well as axonal growth and guidance, triggering behavioral and cognitive abnormalities and dementia (Tay et al. 2018). HDLS, another microgliopathy and a rare autosomal dominant disease, is caused by mutation in tyrosine kinase domain of CSF1R. CSF1R is expressed in microglia and is essential for their development and homeostasis (Rademakers et al. 2012). But how the CSF1R mutation is involved in the disease causation is not known. In vitro studies suggest that DAP12 directly regulate the ability of CSF1R to control the survival and proliferation of macrophages (Otero et al. 2009). This suggests a defect in common signaling pathway in both NHD and HDLS induced by respective mutation in DAP12, TREM2, or CSF1R genes (Tay et al. 2018). Other microglial-related genes implicated in neurological disorders are CD33 in Alzheimer's disease (Hollingworth et al. 2011; Griciuc et al. 2013); TREM2 in frontotemporal dementia (Guerreiro et al. 2013); IRF8 in multiple sclerosis (De Jager et al. 2009); MECP2 gene encoding the transcription repressor methyl-CpG-binding protein 2, in Rett syndrome (Amir et al. 1999; Schafer et al. 2012); and Hoxb in obsessive-compulsive disorder (Chen et al. 2010). Microglial abnormalities/dysfunctions have also been linked with other neurodevelopmental disorders, like autism spectrum disorder (ASD) and obsessive-compulsive disorder (OCD; Morgan et al. 2010; Lee et al. 2017).

Maintenance and expansion of microglia in physiological conditions are solely dependent on their self-renewal ability that is highly susceptible to environmental challenges, stress, and infections during development leading to changes in microglial activity that persists through adulthood. Such alterations in microglial functions have been extensively associated with defects in synaptic maturation and circuitry formation that makes the developing system more vulnerable to mental illness (Paolicelli and Ferretti 2017). This was demonstrated in perinatal immune activation models by inducing viral or bacterial infections in rodents (Giovanoli et al. 2013; Patro et al. 2013; Paolicelli and Ferreti 2017; Baghel et al. 2018; Sarkar et al. 2020), characterized by an increased number of activated microglia and upregulation

of pro-inflammatory cytokines, IL-1β, and TNF-α in the hippocampus. Intermittent and maternal separation stress animal models also suggest the similar changes in microglia leading to the increase in number, density, and surface area of microglia with high phagocytic activity in the hippocampus (Delpech et al. 2016; Paolicelli and Ferretti 2011). Moreover, the microglia remain in prime states, loose their physiological functions, acquire phagocytic phenotype, and show a heightened response to a subsequent stimulus (Diz-Chavas et al. 2013; Paolicelli and Ferretti 2017). These early life adversities leave long-lasting effects in many brain areas, and the pups develop syndrome simulating depression and anxiety (Mintz et al. 2005; Nishi et al. 2014; Sarkar et al. 2019). In all, it appears that the multiple pathogenic factors acting synergistically might be involved in the development of psychiatric diseases (Prinz and Priller 2014). Microglia-mediated neuroinflammation and synaptic defects due to early life adversities have been associated with various human neurological diseases and disorders, viz., periventricular white matter damage, cerebral palsy, autism spectrum disorders, schizophrenia, epilepsy, and perinatal stroke (Prinz and Priller 2014; Paolicelli and Ferretti 2017).

7 Perspectives

The origin and development of microglia have been explored to a great extent; however, a better understanding of the factors involved in the homeostasis, maturation, and guidance of the spatiotemporal specification of microglial phenotypes will enlighten the way for in vitro generation of microglia, expressing typical microglial signature genes and functions similar to CNS microglia. This will be helpful in exploring possibilities and designing protocols for the generation of human microglia live cells from human-induced pluripotent cells for future microglia replacement strategies (Wurm et al. 2021). A detailed understanding of transcriptome and epigenetic changes in microglial activation and microgliopathies will help us to design immunomodulatory therapies for CNS disorders and effective new treatments to combat the neurodevelopmental disturbances.

Acknowledgments The Department of Biotechnology's support through the DBT-National Initiative on Glial Cell Research in Health and Disease is gratefully acknowledged. We are thankful to the Department of Biotechnology, the Department of Science and Technology, and the Indian Council of Medical Research, Govt. of India, for their financial support through various grants. We are thankful to Dr. Urmishree Bedamatta for critically evaluating the manuscript for its English.

References

Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FMV (2011) Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nat Neurosci 14:1142–1149. https://doi.org/10.1038/nn.2887

Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM (2007) Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci 10:1538–1543. https://doi.org/10.1038/nn2014

Akiyama H, McGeer PL (1990) Brain microglia constitutively express β-2 integrins. J Neuroimmunol 30:81–93

Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors originating from yolk sac, and which proliferate in the brain. Brain Res Dev 117:145–152

Alliot F, Lecain E, Grima B, Pessac B (1991) Microglial progenitors with a high proliferative potential in the embryonic and adult mouse brain. Proc Natl Acad Sci U S A 88:1541–1545

Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23:185–188. https://doi.org/10.1038/13810

Appleford PJ, Woollard A (2009) RUNX genes find a niche in stem cell biology. J Cell Biochem 108:14–21

Arno B, Grassivaro F, Rossi C, Bergamaschi A, Castiglioni V, Furlan R, Greter M, Favaro R, Comi G, Becher B, Martino G (2014) Neural progenitor cells orchestrate microglia migration and positioning into the developing cortex. Nat Commun 5:5611

Arnold TD, Lizama CO, Cautivo KM, Santander N, Lin L, Qiu H, Huang EJ, Liu C, Mukouyama YS, Reichardt LF, Zovein AC (2019) Impaired AVβ8 and TGFβ signaling lead to microglial dysmaturation and neuromotor dysfunction. J Exp Med 216:900–915

Arnold TD, Niaudet C, Pang MF, Siegenthaler J, Gaengel K, Jung B, Ferrero GM, Mukouyama YS, Fuxe J, Akhurst R, Betsholtz C (2014) Excessive vascular sprouting underlies cerebral hemorrhage in mice lacking αVβ8-TGFβ signaling in the brain. Development 141:4489–4499

Arnoux I, Audinat E (2015) Fractalkine signaling and microglia functions in the developing brain. Neural Plast 80:43–56. https://doi.org/10.1155/2015/689404

Arnoux I, Hoshiko M, Mandavy L, Avignone E, Yamamoto N, Audinat E (2013) Adaptive phenotype of microglial cells during the normal postnatal development of the somatosensory barrel cortex. Glia 61:1582–1594

Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, Tipton T, Chapman MA, Riecken K, Beccari S, Sierra A (2017) Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. Cell Rep 18:391–405. https://doi.org/10.1016/j.celrep.2016.12.041

Attaai A, Neidert N, von Ehr A, Potru PS, Zöller T, Spittau B (2018) Postnatal maturation of microglia is associated with alternative activation and activated TGFβ signaling. Glia 66:1695–1708

Baghel MS, Singh B, Dhuriya YK, Shukla RK, Patro N, Khanna VK, Patro IK, Thakur MK (2018) Postnatal exposure to poly (I:C) impairs learning and memory through changes in synaptic plasticity gene expression in developing rat brain. Neurobiol Learn Mem 155:379–389

Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, Siklos L, McKercher SR, Appel SH (2006) Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 103:16021–16026

Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, Mulinyawe SB, Bohlen CJ, Adil A, Tucker A, Weissman IL (2016) New tools for studying microglia in the mouse and human CNS. Proc Natl Acad Sci U S A 113:E1738–E1746

Bertrand JY, Jalil A, Klaine M, Jung S, Cumano A, Godin I (2005) Three pathways to mature macrophages in the early mouse yolk sac. Blood 106:3004–3011

Beutner C, Linnartz-Gerlach B, Schmidt SV, Beyer M, Mallmann MR, Staratschek-Jox A, Schultze JL, Neumann H (2013) Unique transcriptome signature of mouse microglia. Glia 61:1429–1442

Bialas AR, Stevens B (2013) TGF-β signaling regulates neuronal C1q expression and developmental synaptic refinement. Nat Neurosci 16:1773–1782

Bianchin MM, Capella HM, Chaves DL, Steindel M, Grisard EC, Ganev GG, da Silva JP, Neto ES, Poffo MA, Walz R, Carlotti CG (2004) Nasu-Hakola disease (polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy—PLOSL): a dementia associated with

bone cystic lesions. From clinical to genetic and molecular aspects. Cell Mol Neurobiol 24:1–24. https://doi.org/10.1023/b:cemn.0000012721.08168.ee
Blank T, Prinz M (2013) Microglia as modulators of cognition and neuropsychiatric disorders. Glia 61:62–70
Bohlen CJ, Bennett FC, Tucker AF, Collins HY, Mulinyawe SB, Barres BA (2017) Diverse requirements for microglial survival, specification, and function revealed by defined-medium cultures. Neuron 94:e8
Böttcher C, Schlickeiser S, Sneeboer MA, Kunkel D, Knop A, Paza E, Fidzinski P, Kraus L, Snijders GJ, Kahn RS, Schulz AR (2019) Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. Nat Neurosci 22:78–90. https://doi.org/10.1038/s41593-018-0290-2
Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B, Wu PM, Doykan CE, Fanek Z (2014) Identification of a unique TGF-b-dependent molecular and functional signature in microglia. Nat Neurosci 17:131–143. https://doi.org/10.1038/nn.3599
Butovsky O, Weiner HL (2018) Microglial signatures and their role in health and disease. Nat Rev Neurosci 19:622–635
Buttgereit A, Lelios I, Yu X, Vrohlings M, Krakoski NR, Gautier EL, Nishinakamura R, Becher B, Greter M (2016) Sall1 is a transcriptional regulator defining microglia identity and function. Nat Immunol 17:1397–1406
Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9:917–924. https://doi.org/10.1038/nn1715
Casano AM, Albert M, Peri F (2016) Developmental apoptosis mediates entry and positioning of microglia in the zebrafish brain. Cell Rep 16:897–906
Chan WY, Kohsaka S, Rezaie P (2007) The origin and cell lineage of microglia: new concepts. Brain Res Rev 53:344–354
Chen JH, Ke KF, Lu JH, Qiu YH, Peng YP (2015) Protection of TGF-β1 against neuroinflammation and neurodegeneration in Aβ1-42-induced Alzheimer's disease model rats. PLoS One 10:49
Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, Capecchi MR (2010) Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. Cell 141:775–785
Chen X, Liu Z, Cao BB, Qiu YH, Peng YP (2017) TGF-β1 neuroprotection via inhibition of microglial activation in a rat model of Parkinson's disease. J Neuroimmune Pharmacol 12:433–446
Cheng LC, Pastrana E, Tavazoie M, Doetsch F (2009) miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. Nat Neurosci 12:399–408
Chihara T, Suzu S, Hassan R, Chutiwitoonchai N, Hiyoshi M, Motoyoshi K, Kimura F, Okada S (2010) IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation. Cell Death Differ 17:1917–1927
Crotti A, Ransohoff RM (2016) Microglial physiology and pathophysiology: insights from genome-wide transcriptional profiling. Immunity 44:505–515
Cuadros M, Navascués J (1998) The origin and differentiation of microglial cells during development. Prog Neurobiol 56:173–189. https://doi.org/10.1016/s0301-0082(98)00035-5
Cumano A, Godin I (2007) Ontogeny of the hematopoietic system. Annu Rev Immunol 25:745–785
Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, Sylvestre V, Stanley ER (2002) Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. Blood 99:111–120
Dalmau I, Vela JM, Gonzalez B, Finsen B, Castellano B (2003) Dynamics of microglia in the developing rat brain. J Comp Neurol 458:144–157

Das Sarma S, Chatterjee K, Dinda H, Chatterjee D, Das Sarma J (2013) Cytomorphological and cytochemical identification of microglia. ISRN Immunol 2013:205431. https://doi.org/10.1155/2013/205431

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8: 752–758

Davoust N, Vuaillat C, Cavillon G, Domenget C, Hatterer E, Bernard A, Dumontel C, Jurdic P, Malcus C, Confavreux C, Belin MF (2006) Bone marrow CD34+/ B220+ progenitors target the inflamed brain and display in vitro differentiation potential toward microglia. The FASEB J 20: 2081–2092

De Biase LM, Schuebel KE, Fusfeld ZH, Jair K, Hawes IA, Cimbro R, Zhang HY, Liu QR, Shen H, Xi ZX, Goldman D (2017) Local cues establish and maintain region-specific phenotypes of basal ganglia microglia. Neuron 95:e6

De Jager PL, Jia X, Wang J, De Bakker PI, Ottoboni L, Aggarwal NT, Piccio L, Raychaudhuri S, Tran D, Aubin C, Briskin R (2009) meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. Nat Genet 41:776–782

Del Río-Hortega P (1919) El "tercer elemento" de los centrosnerviosus. Bol Soc Esp Biol 9:69–120

del Río-Hortega P (1939) The microglia. Lancet 233:1023–1026

Delpech JC, Wei L, Hao J, Yu X, Madore C, Butovsky O, Kaffman A (2016) Early life stress perturbs the maturation of microglia in the developing hippocampus. Brain Behav Immun 57: 79–93. https://doi.org/10.1016/j.bbi.2016.06.006

Diz-Chaves Y, Astiz M, Bellini MJ, Garcia-Segura LM (2013) Prenatal stress increases the expression of proinflammatory cytokines and exacerbates the inflammatory response to LPS in the hippocampal formation of adult male mice. Brain Behav Immun 28:196–206. https://doi.org/10.1016/j.bbi.2012.11.013

Erblich B, Zhu L, Etgen AM, Dobrenis K, Pollard JW (2011) Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. PLoS One 6:e26317

Esiri MM, Al Izzi MS, Reading MC (1991) Macrophages, microglia cells, and HLA-DR antigens in fetal and infant brain. J Clin Pathol 44:102–106

Eyo UB, Miner SA, Weiner JA, Dailey ME (2015) Developmental changes in microglial mobilization are independent of apoptosis in the neonatal mouse hippocampus. Brain Behav Immun 55: 49–59

Fadini GP, Cappellari R, Mazzucato M, Agostini C, De Kreutzenberg SV, Avogaro A (2013) Monocyte-macrophage polarization balance in pre-diabetic individuals. Acta Diabetol 50:977–982. https://doi.org/10.1007/s00592-013-0517-3

Fedoroff S, Zhai R, Novak JP (1997) Microglia and astroglia have a common progenitor cell. J Neurosci Res 50:477–486

Flanders KC, Ludecke GE, Engels SI, Cissel DS, Roberts AB, Kondaiah PA, Lafyatis RO, Sporn MB, Unsicker KL (1991) Localization and actions of transforming growth factor-beta s in the embryonic nervous system. Development 113:183–191

Fujita S, Tsuchihashi Y, Kitamura T (1981) Origin, morphology and function of the microglia. Prog Clin Biol Res 59A:141–169

Gangaraju VK, Lin H (2009) MicroRNAs: key regulators of stem cells. Nat Rev Mol Cell Biol 10: 116–125

Garceau V, Smith J, Paton IR, Davey M, Fares MA, Sester DP, Burt DW, Hume DA (2010) Pivotal advance: avian colony-stimulating factor 1 (CSF-1), interleukin-34 (IL-34), and CSF-1 receptor genes and gene products. J Leukoc Biol 87:753

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841–845

Ginhoux F, Guilliams M (2016) Tissue-resident macrophage ontogeny and homeostasis. Immunity 44:439–449

Ginhoux F, Lim S, Hoeffel G, Low D, Huber T (2013) Origin and differentiation of microglia. Front Cell Neurosci 7:45

Ginhoux F, Prinz M (2018) Origin of microglia: current concepts and past controversies. Cold Spring Harb Perspect Biol 7:a020537

Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, Winter C, Riva MA, Mortensen PB, Feldon J, Schedlowski M (2013) Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. Science 339:1095–1099

Goldmann T, Wieghofer P, Jordão MJ, Prutek F, Hagemeyer N, Frenzel K, Amann L, Staszewski O, Kierdorf K, Krueger M, Locatelli G (2016) Origin, fate and dynamics of macrophages at central nervous system interfaces. Nat Immunol 17:797–805. https://doi.org/10.1038/ni.3423

Gomez-Nicola D, Perry VH (2015) Microglial dynamics and role in the healthy and diseased brain: a paradigm of functional plasticity. Neuroscientist 21:169–184

Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JC, Sajti E, Jaeger BN, O'Connor C, Fitzpatrick C, Pasillas MP, Pena M (2017) An environment-dependent transcriptional network specifies human microglia identity. Science 356:eaal3222

Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, Kündig TM, Frei K, Ginhoux F, Merad M, Becher B (2012) Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. Immunity 37:1050–1060

Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN, Mullin K, Hooli B, Choi SH, Hyman BT, Tanzi RE (2013) Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 78:631–643

Guerreiro RJ, Lohmann E, Brás JM, Gibbs JR, Rohrer JD, Gurunlian N, Dursun B, Bilgic B, Hanagasi H, Gurvit H, Emre M (2013) Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement. JAMA Neurol 70:78–84

Hagemeyer N, Hanft KM, Akriditou MA, Unger N, Park ES, Stanley ER, Staszewski O, Dimou L, Prinz M (2017) Microglia contribute to Normal Myelinogenesis and to oligodendrocyte progenitor maintenance during adulthood. Acta Neuropathol 134:441–458

Hagemeyer N, Kierdorf K, Frenzel K, Xue J, Ringelhan M, Abdullah Z, Godin I, Wieghofer P, Costa Jordão MJ, Ulas T, Yorgancioglu G (2016) Transcriptome-based profiling of yolk sac-derived macrophages reveals a role for Irf8 in macrophage maturation. EMBO J 35:1730–1744

Hamby ME, Hewett JA, Hewett SJ (2010) Smad3-dependent signaling underlies the TGF-beta1–mediated enhancement in astrocytic iNOS expression. Glia 58:1282–1291

Hao C, Richardson A, Fedoroff S (1991) Macrophage-like cells originate from neuroepithelium in culture: characterization and properties of the macrophage-like cells. Int J Dev Neurosci 9:1–14

Harry GJ (2013) Microglia during development and aging. Pharmacol Ther 139(3):313–326

Harry GJ, Kraft AD (2012) Microglia in the developing brain: A potential target with lifetime effects. Neurotoxicology 33:191–206

Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, Becker CD, See P, Price J, Lucas D, Greter M (2013) Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 38:792–804

Heinz S, Romanoski CE, Benner C, Glass CK (2015) The selection and function of cell type-specific enhancers. Nat Rev Mol Cell Biol 16:144–154

Herbomel P, Thisse B, Thisse C (1999) Ontogeny and behaviour of early macrophages in the zebrafish embryo. Development 126:3735–3745

Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, Beaudin AE, Lum J, Low I, Forsberg EC, Poidinger M (2015) C-Myb (1) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. Immunity 42:665–678

Hoeffel G, Ginhoux F (2015) Ontogeny of tissue-resident macrophages. Front Immunol 6:486

Holland R, McIntosh AL, Finucane OM, Mela V, Rubio-Araiz A, Timmons G, McCarthy SA, Gun'ko YK, Lynch MA (2018) Inflammatory microglia are glycolytic and iron retentive and

typify the microglia in APP/PS1 mice. Brain Behav Immun 68:183–196. https://doi.org/10.1016/j.bbi.2017.10.017
Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43:429–435
Holtman IR, Raj DD, Miller JA, Schaafsma W, Yin Z, Brouwer N, Wes PD, Möller T, Orre M, Kamphuis W, Hol EM (2015) Induction of a common microglia gene expression signature by aging and neurodegenerative conditions: a co-expression meta-analysis. Acta Neuropathol Commun 3:31
Holtschke T, Lohler J, Kanno Y, Fehr T, Giese N, Rosenbauer F, Lou J, Knobeloch KP, Gabriele L, Waring JF, Bachmann MF (1996) Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell 87:307–317
Hoshiko M, Arnoux I, Avignone E, Yamamoto N, Audinat E (2012) Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. J Neurosci 32:15106–15111. https://doi.org/10.1523/JNEUROSCI.1167-12.2012
Hristova M, Cuthill D, Zbarsky V, Acosta-Saltos A, Wallace A, Blight K, Buckley SM, Peebles D, Heuer H, Waddington SN, Raivich G (2010) Activation and deactivation of periventricular white matter phagocytes during postnatal mouse development. Glia 58:11–28
Huang G, Zhang P, Hirai H, Elf S, Yan X, Chen Z, Koschmieder S, Okuno Y, Dayaram T, Growney JD, Shivdasani RA (2008) PU.1 is a major downstream target of AML1 (RUNX1) in adult mouse haematopoiesis. Nat Genet 40:51–60
Hughes V (2012) Microglia: the constant gardeners. Nature 485:570–572
Hume DA, Perry VH, Gordon S (1983) Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: Phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. J Cell Biol 97:253–257
Jenkins SJ, Ruckerl D, Thomas GD, Hewitson JP, Duncan S, Brombacher F, Maizels RM, Hume DA, Allen JE (2013) IL-4 directly signals tissue-resident macrophages to proliferate beyond homeostatic levels controlled by CSF-1. J Exp Med 210:2477–2491. https://doi.org/10.1084/jem.20121999
Jeong HK, Ji K, Min K, Joe EH (2013) Brain inflammation and microglia: facts and misconceptions. Exp Neurobiol 22:59–67. https://doi.org/10.5607/en.2013.22.2.59
Jones BA, Beamer M, Ahmed S (2010) Fractalkine/CX3CL1: a potential new target for inflammatory diseases. Mol Interv 10:263–270. https://doi.org/10.1124/mi.10.5.3
Jurga AM, Paleczna M, Kuter KZ (2020) Overview of general and discriminating markers of differential microglia phenotypes. Front Cell Neurosci 14:198. https://doi.org/10.3389/fncel.2020.00198
Kagoshima H, Shigesada K, Kohara Y (2007) RUNX regulates stem cell proliferation and differentiation: insights from studies of C. elegans. J Cell Biochem 100:1119–1130
Kaifu T, Nakahara J, Inui M, Mishima K, Momiyama T, Kaji M, Sugahara A, Koito H, Ujike-Asai A, Nakamura A, Kanazawa K, Tan-Takeu-Chi K, Iwasaki K, Yokoyama WM, Kudo A, Fujiwara M, Asou H, Takai T (2003) Osteopetrosis and thalamic hypomyelinosis with synaptic degeneration in DAP12-deficient mice. J Clin Invest 111:323–332
Kaur C, Ling EA (1991) A study of the transformation of amoeboid microglial cells into microglia labelled with the isolectin Griffonia simplicifolia in postnatal rats. Acta Anat 142:118–125
Kaur C, Ling EA, Wong WC (1987) Localisation of thiamine pyrophosphatase in the amoeboid microglial cells in the brain of postnatal rats. J Anat 152:13–22
Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, Wieghofer P, Heinrich A, Riemke P, Hölscher C, Müller DN (2013) Microglia emerge from erythromyeloid precursors via Pu.1- and Irf-8-dependent pathways. Nat Neurosci 16:273–280
Kostović I, Judas M (2002) Correlation between the sequential ingrowth of afferents and transient patterns of cortical lamination in preterm infants. Anat Rec 267:1–6

Koushik SV, Wang J, Rogers R, Moskophidis D, Lambert NA, Creazzo TL, Conway SJ (2001) Targeted inactivation of the sodium-calcium exchanger (Ncx1) results in the lack of a heartbeat and abnormal myofibrillar organization. FASEB J 15:1209–1211. https://doi.org/10.1096/fj.00-0696fje

Kurz H, Korn J, Eggli PS, Huang R, Christ B (2001) Embryonic central nervous system angiogenesis does not involve blood-borne endothelial progenitors. J Comp Neurol 436:263–274

Lagasse E, Weissman IL (1997) Enforced expression of Bcl-2 in monocytes rescues macrophages and partially reverses osteopetrosis in op/op mice. Cell 89:1021–1031

Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39:151–170. https://doi.org/10.1016/0306-4522(90)90229-w

Lee AS, Azmitia EC, Whitaker-Azmitia PM (2017) Developmental microglial priming in postmortem autism spectrum disorder temporal cortex. Brain Behav Immun 62:193–202

Lehnardt S (2010) Innate immunity and neuroinflammation in the CNS: the role of microglia in Toll-like receptor-mediated neuronal injury. Glia 58:253–263

Lelli A, Gervais A, Colin C, Chéret C, de Almodovar CR, Carmeliet P, Krause KH, Boillée S, Mallat M (2013) The NADPH oxidase Nox2 regulates VEGFR1/CSF-1R-mediated microglial chemotaxis and promotes early postnatal infiltration of phagocytes in the subventricular zone of the mouse cerebral cortex. Glia 61:1542–1555. https://doi.org/10.1002/glia.22540

Lenz KM, Nelson LH (2018) Microglia and beyond: innate immune cells as regulators of brain development and behavioral function. Front Immunol 9:698. https://doi.org/10.3389/fimmu.2018.00698

Lin HH, Faunce DE, Stacey M, Terajewicz A, Nakamura T, Zhang-Hoover J, Kerley M, Mucenski ML, Gordon S, Stein-Streilein J (2005) The macrophage F4/80 receptor is required for the induction of antigen-specific efferent regulatory T cells in peripheral tolerance. J Exp Med 201:1615–1625. https://doi.org/10.1084/jem.20042307

Ling EA (1981) The origin and nature of microglia. Adv Cell Neurobiol 2:33–82

Ling EA (1994) Monocytic origin of ramified microglia in the corpus callosum in postnatal rat. Neuropathol Appl Neurobiol 20:182–183

Ling EA, Tan CK (1974) Amoeboid microglial cells in the corpus callosum of neonatal rats. Arch Histol Jpn 36:265–280

Liu Y, Aguzzi A (2019) NG2 glia are required for maintaining microglia homeostatic state. Glia 68:345–355

Lopez-Atalaya JP, Askew KE, Sierra A, Gomez-Nicola D (2018) Development and maintenance of the brain's immune toolkit: microglia and nonparenchymal brain macrophages. Dev Neurobiol 78(6):561–579

Lyons A, Downer EJ, Crotty S, Nolan YM, Mills KHG, Lynch MA (2007) CD200 ligand receptor interaction modulates microglial activation in vivo and in vitro: a role for IL-4. J Neurosci 27:8309–8313. https://doi.org/10.1523/JNEUROSCI.1781-07.2007

Marin-Teva JL, Almendros A, Calvente R, Cuadros MA, Navascues J (1999) Proliferation of actively migrating ameboid microglia in the developing quail retina. Anat Embryol 200:289–300

Marin-Teva JL, Dusart I, Colin C, Gervais A, Mallat M (2004) Microglia promote the death of developing Purkinje cells. Neuron 41:535–547

Mass E, Ballesteros I, Farlik M, Halbritter F, Günther P, Crozet L, Jacome-Galarza CE, Händler K, Klughammer J, Kobayashi Y, Gomez-Perdiguero E (2016) Specification of tissue-resident macrophages during organogenesis. Science 353:5

Masuda T, Amann L, Sankowski R, Staszewski O, Lenz M, Snaidero N, Jordão MJ, Böttcher C, Kierdorf K, Jung S, Priller J (2020a) Novel Hexb-based tools for studying microglia in the CNS. Nat Immunol 21:802–815

Masuda T, Sankowski R, Staszewski O, Böttcher C, Amann L, Scheiwe C, Nessler S, Kunz P, van Loo G, Coenen VA, Reinacher PC (2019) Spatial and temporal heterogeneity of mouse and

human microglia at single-cell resolution. Nature 566:388–392. https://doi.org/10.1038/s41586-019-0924-x
Masuda T, Sankowski R, Staszewski O, Prinz M (2020b) Microglia heterogeneity in the single-cell era. Cell Rep 30:1271–1281. https://doi.org/10.1016/j.celrep.2020.01.010
Matcovitch-Natan O, Winter DR, Giladi A, Aguilar SV, Spinrad A, Sarrazin S, Ben-Yehuda H, David E, González FZ, Perrin P, Keren-Shaul H (2016) Microglia development follows a stepwise program to regulate brain homeostasis. Science 353:aad8670
Metcalf D (1985) The granulocyte-macrophage colony-stimulating factors. Science 229:16–22
Minghetti L, Levi G (1998) Microglia as effector cells in brain damage and repair: focus on prostanoids and nitric oxide. Prog Neurobiol 54:99–125
Minten C, Terry R, Deffrasnes C, King NJ, Campbell IL (2012) IFN regulatory factor 8 is a key constitutive determinant of the morphological and molecular properties of microglia in the CNS. PLoS One 7:e49851
Mintz M, Ruedi-Bettschen D, Feldon J, Pryce CR (2005) Early social and physical deprivation leads to reduced social motivation in adulthood in Wistar rats. Behav Brain Res 156:311–320. https://doi.org/10.1016/j.bbr.2004.08.017
Monier A, Dle-Biassette H, Delezoide AL, Evrard P, Gressens P, Verney C (2007) Entry and distribution of microglial cells in human embryonic and foetal cerebral cortex. J Neuropathol Exp Neurol 66:372–382
Monier A, Evrard P, Gressens P, Verney C (2006) Distribution and differentiation of microglia in the human encephalon during the first two trimesters of gestation. J Comp Neurol 499:565–582
Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, Courchesne E, Everall IP (2010) Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry 68:368–376
Mosser CA, Baptista S, Arnoux I, Audinat E (2017) Microglia in CNS development: shaping the brain for future. Prog Neurobiol 149-150:1–20
Moujahid A, Navascués J, Marín-Teva JL, Cuadros MA (1996) Macrophages during avian optic nerve development: relationship to cell death and differentiation into microglia. Anat Embryol 193:131–144
Mukherjee S, Mukherjee S, Bhattacharya S, Babu SPS (2016) Surface proteins of Setaria cervi induces inflammation in macrophage through Toll-like Receptor 4 (TLR4) mediated signaling pathway. Parasite Immunol 39:e12389. https://doi.org/10.1111/pim.12389
Murabe Y, Sano Y (1982) Morphological studies on neuroglia: VI. Postnatal development of microglial cells. Cell Tissue Res 225:469–485
Murabe Y, Sano Y (1983) Morphological studies on neuroglia: VII. Distribution of "brain macrophages" in brains of neonatal and adult rats, as determined by means of immunohistochemistry. Cell Tissue Res 229:85–95
Naito M, Takahashi K, Nishikawa S (1990) Development, differentiation, and maturation of macrophages in the fetal mouse liver. J Leukoc Biol 48:27–37
Naito M, Umeda S, Yamamoto T, Moriyama H, Umezu H, Hasegawa G, Usuda H, Shultz LD, Takahashi K (1996) Development, differentiation, and phenotypic heterogeneity of murine tissue macrophages. J Leukoc Biol 59:133–138
Nataf S, Anginot A, Vuaillat C, Malaval L, Fodil N, Chereul E, Langlois JB, Dumontel C, Cavillon G, Confavreux C, Mazzorana M (2005) Brain and bone damage in KARAP/DAP12 loss-of-function mice correlate with alterations in microglia and osteoclast lineages. Am J Pathol 166:275–286
Navascues J, Calvente R, Marin-Teva JL, Cuadros MA (2000) Entry, dispersion and differentiation of microglia in the developing central nervous system. An Acad Bras Cienc 72:91–102
Nayak D, Roth TL, McGavern DB (2014) Microglia development and function. Annu Rev Immunol 32:367–402
Neumann H, Takahashi K (2007) Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis. J Neuroimmunol 184:92–99

Nikodemova M, Kimyon RS, De I, Small AL, Collier LS, Watters JJ (2015) Microglial numbers attain adult levels after undergoing a rapid decrease in cell number in the third postnatal week. J Neuroimmunol 278:280–288. https://doi.org/10.1016/j.jneuroim.2014.11.018

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318

Nishi M, Horii-Hayashi N, Sasagawa T (2014) Effects of early life adverse experiences on the brain: implications from maternal separation models in rodents. Front Neurosci 8:166. https://doi.org/10.3389/fnins.2014.00166

Olson MC, Scott EW, Hack AA, Su GH, Tenen DG, Singh H, Simon MC (1995) PU.1 is not essential for early myeloid gene expression but is required for terminal myeloid differentiation. Immunity 3:703–714

Orlowski D, Soltys Z, Janeczko K (2003) Morphological development of microglia in the postnatal rat brain. Int J Dev Neurosci 21:445–450

Otero K, Turnbull IR, Poliani PL, Vermi W, Cerutti E, Aoshi T, Tassi I, Takai T, Stanley SL, Miller M, Shaw AS (2009) Macrophage colony-stimulating factor induces the proliferation and survival of macrophages via a pathway involving DAP12 and β-catenin. Nat Immunol 10:734–743

Palis J, Robertson S, Kennedy M, Wall C, Keller G (1999) Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development 126:5073–5084

Paloneva J, Autti T, Raininko R, Partanen J, Salonen O, Puranen M, Hakola P, Haltia M (2001) CNS manifestations of Nasu-Hakola disease: a frontal dementia with bone cysts. Neurology 56:1552–1558. https://doi.org/10.1212/WNL.56.11.1552

Paloneva J, Kestilä M, Wu J, Salminen A, Böhling T, Ruotsalainen V, Hakola P, Bakker AB, Phillips JH, Pekkarinen P, Lanier LL, Timonen T, Peltonen L (2000) Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. Nat Genet 25:357–361. https://doi.org/10.1038/77153

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D (2011) Synaptic pruning by microglia is necessary for Normal brain development. Science 333:1456–1458

Paolicelli RC, Ferretti MT (2017) Function and dysfunction of microglia during brain development: consequences for synapses and neural circuits. Front Synap Neurosci 9:9. https://doi.org/10.3389/fnsyn.2017.00009

Pasciuto E, Burton OT, Roca CP, Lagou V, Rajan WD, Theys T, Mancuso R, Tito RY, Kouser L, Callaerts-Vegh Z, Alerie G (2020) Microglia require CD4 T cells to complete the fetal-to-adult transition. Cell 182:625–640.e24

Patro N, Singh K, Patro I (2013) Differential microglial and astrocytic response to bacterial and viral infection in the developing hippocampus of neonatal rats. Indian J Exp Biol 51:606–614

Perdiguero EG, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, Garner H, Trouillet C, De Bruijn MF, Geissmann F, Rodewald HR (2015) Tissue resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature 518:547–551

Perry VH, Hume DA, Gordon S (1985) Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience 15:313–326

Perry VH, Nicoll JAR, Holmes C (2010) Microglia in neurodegenerative disease. Nat Rev Neurol 6:193–201

Pixley FJ, Stanley ER (2004) CSF-1 regulation of the wandering macrophage: complexity in action. Trends Cell Biol 14:628–638

Ponomarev ED, Veremeyko T, Barteneva N, Krichevsky AM, Weiner HL (2011) MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU.1 pathway. Nat Med 17:64–70

Pont-Lezica L, Bechade C, Belarif-Cantaut Y, Pascual O, Bessis A (2011) Physiological roles of microglia during development. J Neurochem 119:901–908

Pont-Lezica L, Beumer W, Colasse S, Drexhage H, Versnel M, Bessis A (2014) Microglia shape corpus callosum axon tract fasciculation: functional impact of prenatal inflammation. Eur J Neurosci 39:1551–1557. https://doi.org/10.1111/ejn.12508

Prinz M, Erny D, Hagemeyer N (2017) Ontogeny and homeostasis of CNS myeloid cells. Nat Immunol 18:385–392

Prinz M, Priller J (2014) Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. Nat Rev Neurosci 15:300–312. https://doi.org/10.1038/nrn3722

Rademakers R, Baker M, Nicholson AM, Rutherford NJ, Finch N, Soto-Ortolaza A, Lash J, Wider C, Wojtas A, DeJesus-Hernandez M, Adamson J (2012) Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. Nat Genet 44:200–205

Rakic S, Zecevic N (2000) Programmed cell death in the developing human telencephalon. Eur J Neurosci 12:2721–2734

Ransohoff RM (2011) Microglia and monocytes: 'tis plain the twain meet in the brain. Nat Neurosci 14:1098–1100. https://doi.org/10.1038/nn.2917

Ransohoff RM, Perry VH (2009) Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol 27:119–145

Réu P, Khosravi A, Bernard S, Mold JE, Salehpour M, Alkass K, Perl S, Tisdale J, Possnert G, Druid H, Frisén J (2017) The lifespan and turnover of microglia in the human brain. Cell Rep 20: 779–784

Rezaie P (2003) Microglia in the human nervous system during development. Neuroembryology 2: 18–31. https://doi.org/10.1159/000068498

Rezaie P, Dean A, Male D, Ulfig N (2005) Microglia in the cerebral wall of the human telencephalon at second trimester. Cereb Cortex 15:938–949

Rezaie P, Male D (2003) Microglia in fetal and adult human brain can be distinguished from other mononuclear phagocytes through their lack of CD163 expression. Neuroembryology 2:130–133

Rezaie P, Patel K, Male D (1999) Microglia in the human fetal spinal cord—patterns of distribution, morphology and phenotype. Dev Brain Res 115:71–81. https://doi.org/10.1016/s0165-3806(99)00043-7

Rezaie P, Trillo-Pazos G, Greenwood J, Everall IP, Male DK (2002) Motility and ramification of human fetal microglia in culture: an investigation using time-lapse video microscopy and image analysis. Exp Cell Res 274:68–82

Rice RA, Pham J, Lee RJ, Najafi AR, West BL, Green KN (2017) Microglial repopulation resolves inflammation and promotes brain recovery after injury. Glia 65:931–944. https://doi.org/10.1002/glia.23135

Rigato C, Buckinx R, Le-Corronc H, Rigo JM, Legendre P (2011) Pattern of invasion of the embryonic mouse spinal cord by microglial cells at the time of the onset of functional neuronal networks. Glia 59:675–695

Rigato C, Swinnen N, Buckinx R, Couillin I, Mangin JM, Rigo JM, Legendre P, Le CH (2012) Microglia proliferation is controlled by P2 7 receptors in a pannexin-1-independent manner during early embryonic spinal cord invasion. J Neurosci 32:11559–11573

Rosenbauer F, Tenen DG (2007) Transcription factors in myeloid development: balancing differentiation with transformation. Nat Rev Immunol 7:105–117

Roumier A, Béchade C, Poncer JC, Smalla KH, Tomasello E, Vivier E, Gundelfinger ED, Triller A, Bessis A (2004) Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse. J Neurosci 24:11421–11428

Samokhvalov IM, Samokhvalova NI, Nishikawa S (2007) Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. Nature 446:1056–1061

Sarkar T, Patro N, Patro IK (2019) Cumulative multiple early life hits- a potent threat leading to neurological disorders. Brain Res Bull 147:58–68

Sarkar T, Patro N, Patro IK (2020) Neuronal changes and cognitive deficits in a multi-hit rat model following cumulative impact of early life stressors. Biology Open 9:bio054130. https://doi.org/10.1242/bio.054130

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74:691–705

Schlegelmilch T, Henke K, Peri F (2011) Microglia in the developing brain: from immunity to behaviour. Curr Opin Neurobiol 21:5–10

Schulz C, Perdiguero EG, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW, Frampton J (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336:86–90

Shechter R, London A, Varol C, Raposo C, Cusimano M, Yovel G, Rolls A, Mack M, Pluchino S, Martino G, Jung S (2009) Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. PLoS Med 6:e10000113

Shechter R, Miller O, Yovel G, Rosenzweig N, London A, Ruckh J, Kim KW, Klein E, Kalchenko V, Bendel P, Lira SA (2013) Recruitment of beneficial M2 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. Immunity 38:555–569

Sheng J, Ruedl C, Karjalainen K (2015) Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. Immunity 43:382–393

Shiau CE, Kaufman Z, Meireles AM, Talbot WS (2015) Differential requirement for irf8 in formation of embryonic and adult macrophages in zebrafish. PLoS One 10:e0117513

Shklover J, Levy-Adam F, Kurant E (2015) Apoptotic cell clearance in development. Curr Top Dev Biol 2015:297–334

Shrivastava K, Gonzalez P, Acarin L (2012) The immune inhibitory complex CD200/CD200R is developmentally regulated in the mouse brain. J Comp Neurol 520:2657–2675. https://doi.org/10.1002/cne.23062

Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Annunziata N (1992) Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature 359:693–699

Smith AM, Gibbons HM, Oldfield RL, Bergin PM, Mee EW, Faull RL, Dragunow M (2013) ThetranscriptionfactorPU.1 is critical for viability and function of human brain microglia. Glia 61:929–942

Sorokin SP, Hoyt RF Jr, Blunt DG, McNelly NA (1992) Macrophage development: II. Early ontogeny of macrophage populations in brain, liver and lungs of rat embryos as revealed by a lectin marker. Anat Rec 232:527–550. https://doi.org/10.1002/ar.1092320410

Spassky N, Le BB HK, Queraud-Le Saux F, Bloch-Gallego E, Chedotal A, Zalc B, Thomas JL (2002) Directional guidance of oligodendroglial migration by class 3 semaphorins and netrin-1. J Neurosci 22:5992–6004

Spittau B, Dokalis N, Prinz M (2020) The role of TGFβ signaling in microglia maturation and activation. Trends Immunol 41:836–848

Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AM, Lambris JD, Smith SJ, John SW, Barres BA (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131:1164–1178

Swinnen N, Smolders S, Avila A, Notelaers K, Paesen R, Ameloot M, Brône B, Legendre P, Rigo JM (2013) Complex invasion pattern of the cerebral cortex by microglial cells during development of the mouse embryo. Glia 61:150–163

Takahashi K, Naito M (1993) Development, differentiation, and proliferation of macrophages in the rat yolk sac. Tissue Cell 25:351–362

Takahashi K, Rochford CD, Neumann H (2005) Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med 201:647–657. https://doi.org/10.1084/jem.20041611

Taniguchi T, Ogasawara K, Takaoka A, Tanaka N (2001) IRF family of transcription factors as regulators of host defense. Annu Rev Immunol 19:623–655

Tay TL, Bechade C, D'Andrea I, St Pierre MK, Henry MS, Roumier A, Tremblay ME (2018) Microglia gone rogue: impacts on psychiatric disorders across the lifespan. Front Mol Neurosci 10:421

Tay TL, Savage JC, Hui CW, Bisht K, Tremblay ME (2017) Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. J Physiol 595(6):1929–1945

Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, Yamashita T (2013) Layer V cortical neurons require microglial support for survival during postnatal development. Nat Neurosci 16: 543–551. https://doi.org/10.1038/nn.3358

Umpierre AD, Wu L-J (2020) Microglia research in the 100th year since its discovery. Neurosci Bull 36:303–306

Upender MB, Naegele JR (1999) Activation of microglia during developmentally regulated cell death in the cerebral cortex. Dev Neurosci 21:491–505

Vainchtein ID, Chin G, Cho FS, Kelley KW, Miller JG, Chien EC, Liddelow SA, Nguyen PT, Nakao-Inoue H, Dorman LC, Akil O (2018) Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development. Science 359:1269–1273

Van Ryzin JW, Pickett LA, McCarthy MM (2018) Microglia: driving critical periods and sexual differentiation of the brain. Dev Biol 78(6):580–592

Varol D, Mildner A, Blank T, Shemer A, Barashi N, Yona S, David E, Boura-Halfon S, Segal-Hayoun Y, Chappell-Maor L, Keren-Shaul H (2017) Dicer deficiency differentially impacts microglia of the developing and adult brain. Immunity 46(1030–1044):e8

Verney C, Monier A, Fallet-Bianco C, Gressens P (2010) Early microglial colonization of the human forebrain and possible involvement in periventricular white-matter injury of preterm infants. J Anat 217:436–448

Wakselman S, Béchade C, Roumier A, Bernard D, Triller A, Bessis A (2008) Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. J Neurosci 28:8138–8143

Walls JR, Coultas L, Rossant J, Henkelman RM (2008) Three-dimensional analysis of vascular development in the mouse embryo. PLoS One 5:e2853

Walton MR, Gibbons H, MacGibbon GA, Sirimanne E, Saura J, Gluckman PD, Dragunow M (2000) PU.1 expression in microglia. J Neuroimmunol 104:109–115

Wang CC, Wu CH, Shieh JH, Wen CY (2002) Microglial distribution and apoptosis in fetal rat brain. Dev Brain Res 139:337–342

Wang X, Zhao L, Zhang J, Fariss RN, Ma W, Kretschmer F, Wang M, Qian H, Badea TC, Diamond JS, Gan WB (2016) Requirement for microglia for the maintenance of synaptic function and integrity in the mature retina. J Neurosci 36:2827–2842

Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, Barrow AD, Diamond MS, Colonna M (2012) IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat Immunol 13:753–760

Wu CH, Wen CY, Shieh JY, Ling EA (1994) Down-regulation of membrane glycoprotein in amoeboid microglia transforming into ramified microglia in postnatal rat brain. J Neurocytol 23: 258–269

Wurm J, Konttinen H, Andressen C, Malm T, Spittau B (2021) Microglia development and maturation and its implications for induction of microglia-like cells from human iPSCs. Int J Mol Sci 22:3088

Xu J, Wang T, Wu Y, Jin W, Wen Z (2016) Microglia colonization of developing zebrafish midbrain is promoted by apoptotic neuron and lysophosphatidylcholine. Dev Cell 38:214–222. https://doi.org/10.1016/j.devcel.2016.06.018

Yu JY, Chung KH, Deo M, Thompson RC, Turner DL (2008) MicroRNA miR-124 regulates neurite outgrowth during neuronal differentiation. Exp Cell Res 314:2618–2633

Yun SP, Kam TI, Panicker N, Kim S, Oh Y, Park JS, Kwon SH, Park YJ, Karuppagounder SS, Park H, Kim S (2018) Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. Nat Med 24:931–938. https://doi.org/10.1038/s41591-018-0051-5

Zanoni I, Ostuni R, Marek LR, Barresi S, Barbalat R, Barton GM, Granucci F, Kagan JC (2011) CD14 controls the LPS-induced endocytosis of toll-like receptor 4. Cell 147:868–880. https://doi.org/10.1016/j.cell.2011.09.051

Zhang DE, Hetherington CJ, Meyers S, Rhoades KL, Larson CJ, Chen HM, Hiebert SW, Tenen DG (1996) CCAAT enhancer-binding protein (C/EBP) and AML1 (CBF alpha2) synergistically activate the macrophage colony-stimulating factor receptor promoter. Mol Cell Biol 16:1231–1240

Zöller T, Schneider A, Kleimeyer C, Masuda T, Potru PS, Pfeifer D, Blank T, Prinz M, Spittau B (2018) Silencing of TGFβ signalling in microglia results in impaired homeostasis. Nat Commun 9:4011

Zusso M, Methot L, Lo R, Greenhalgh AD, David S, Stifani S (2012) Regulation of postnatal forebrain amoeboid microglial cell proliferation and development by the transcription factor Runx1. J Neurosci 32:11285–11298

Biology of Astrocytes in CNS Infection

Radhika Mhatre, Anita Mahadevan, and S. K. Shankar

Abstract

There is an increasing interest in the central role of astrocytes in CNS injury and disease. They react to injury by the increased expression of a variety of proteins, which causes their transformation into "reactive" astrocytes, a state with specific structural and functional characteristics. The responses of reactive astrocytes vary in a context-dependent manner. Reactive astrocytes release a wide variety of extracellular molecules, including inflammatory modulators, chemokines and cytokines, and various neurotrophic factors that can be either neuroprotective or neurotoxic, influencing neural function, regulation of blood flow, synaptic function, and plasticity. In CNS pathologies, reactive astrocytes represent a double-edged sword; on the one hand, they worsen the extent of injury due to the release of pro-apoptotic substances and Ca2+, while, on the other hand, they contribute to regenerative processes during the chronic phase of injury. The interplay between the neuroprotective and neurotoxic effects of reactive gliosis determines effect on cognitive function. Unravelling the variety of signaling pathways that modulate astrocyte activation may contribute to the development of novel therapeutic strategies that could decrease the negative influence of astrocytes while enhancing their positive effect on the outcome of pathological processes.

In a detailed morphologic and morphometric study of a large number of cases of CNS infections (including tuberculous meningitis and cryptococcal meningitis) diagnosed and archived at our center, the alterations in microglia and astrocytes were evaluated. In tuberculous meningitis, the activated microglia and astrocytes displayed hypertrophy and hyperplasia, aggregating in proximity to the meningeal exudates. In cases of cryptococcal meningitis, reactive changes

R. Mhatre · A. Mahadevan (✉) · S. K. Shankar
Department of Neuropathology, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, Karnataka, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_8

were less prominent, though activation of both cellular elements was found. Association of HIV with these opportunistic infections resulted in muted glial and microglial response. The reactive astrocytes and microglia following opportunistic infection developed dystrophic changes heralding senescence. Both astroglial and microglial cells also expressed caspase-3, a pro-apoptotic marker, following HIV and opportunistic infections. Neurocognitive sequelae are increasingly being recognized as long terms sequelae of survivors of CNS infections. It is tempting to speculate that blocking the astrocytes' metamorphosis into toxic cells or pharmacological blockade of toxic substances might modulate/prevent the long-term neurocognitive sequelae in survivors. Teasing apart these complex cellular interactions remains an important step towards fully understanding the molecular pathogenesis of CNS-infections. This will help translate to rational design of new medicines and therapeutic vaccines.

Keywords

Reactive astrogliosis · Meningitis · Encephalitis · Abscess

1 Introduction

Neurocognitive sequelae are increasingly being recognized as long terms consequences in survivors of CNS infections. Astrocytes the most abundant cell type in the CNS can be both infected by or act as responder to infectious agents. Astrocytes are known to integrate numerous homeostatic functions in the CNS, and their infection inevitably affects neighboring cells, especially neurons. Astrocytes regulate innate and adaptive immune responses in the CNS under pathological conditions. Following antigen recognition, astrocytes participate in the initiation of innate immune responses and prompt an adaptive immune response to recruit peripheral immune cells (Geyer et al. 2019). They react to injury by the increased expression of a variety of proteins, which causes their transformation into "reactive" astrocytes, a state with specific structural and functional characteristics. The responses of reactive astrocytes vary in a context-dependent manner. Reactive astrocytes release a wide variety of extracellular molecules, including inflammatory modulators, chemokines and cytokines, and various neurotrophic factors that can be either neuroprotective or neurotoxic, influencing neural function, regulation of blood flow, synaptic function, and plasticity. In CNS pathologies, reactive astrocytes represent a double-edged sword; on the one hand, they worsen the extent of injury due to the release of pro-apoptotic substances and Ca2+, while, on the other hand, they contribute to regenerative processes during the chronic phase of injury. The interplay between the neuroprotective and neurotoxic effects of reactive gliosis determines effect on cognitive function. Unravelling the variety of signaling pathways that modulate astrocyte activation may contribute to the development of novel therapeutic strategies that could mitigate the negative influence of astrocytes while enhancing their positive effect on the outcome of pathological processes.

2 Reactive Astrogliosis

Reactive astrogliosis includes a spectrum of changes that occur in response to all forms and severities of CNS injury and disease, along a gradated continuum regulated in a context-specific manner by inter- and intracellular signaling molecules. These have the potential to alter astrocyte activities, either through gain or loss of functions that can impact either in a beneficial or detrimental manner on the surrounding neural and nonneural cells (Sofroniew 2009). Astrocytes are ubiquitously present in the CNS in nonoverlapping domains within the gray matter. Similar domains are also hypothesized to exist in the white matter, although not extensively studied. In healthy CNS tissue, not all astrocytes express detectable levels of glial fibrillary acidic protein (GFAP). Reactive astrogliosis is a defense mechanism which fulfils the following actions: isolate the damaged area from the rest of the CNS tissue, reconstruct the damaged blood brain barrier, and facilitate the remodeling of brain circuits in areas surrounding the lesion (Raivich et al. 1999). The biochemical hallmark of reactive astrogliosis is the upregulation of the synthesis of intermediate filament proteins GFAP and vimentin. Brain damage very rapidly transforms most of the astroglial cells into GFAP-positive reactive astrocytes. Reactive astrogliosis is classified into three broad categories which transition along a graded continuum.

Mild to moderate reactive astrogliosis: It is characterized by upregulation of GFAP and its expression by most astrocytes, along with hypertrophy of cell body and processes within the individual domains, with nonoverlapping processes. If the trigger is withdrawn, this form can undergo resolution (Sofroniew and Vinters 2010). In the context of infection, this occurs with diffuse innate immune activation following viral or bacterial infections.

Severe diffuse reactive astrogliosis: This form is depicted by prominent up regulation and expression of GFAP, along with proliferation of astrocytes. There is no respect for individual domains with overlapping of astrocytic processes. It is associated with substantial restructuring of tissue architecture which persists after withdrawal of stimulus (Sofroniew and Vinters 2010). It is seen surrounding focal infectious process.

Severe reactive astrogliosis with compact glial scar formation: Formation of dense compact glial scar results due to marked overlapping of astrocyte processes and disruption of domains. Scars form along the borders of severe tissue damage and serve as neuroprotective barriers to inflammatory cells and infectious agents. The scar is reinforced by fibromeningeal and other glial cells with deposition of a dense collagenous extracellular matrix (Sofroniew and Vinters 2010; Voskuhl et al. 2009). Obvious example of this process is the capsule surrounding an abscess.

3 Subtypes of Astrocytes

Morphologic heterogeneity within astrocytes was first described by Cajal over a century ago. Based on the structure and anatomic location, astroglia have been broadly categorized as protoplasmic astrocytes in the gray matter and fibrous

astrocytes in the white matter (John Lin et al. 2017; Miller 2018). Another morphological change in astrocytes seen in either reactive or neoplastic conditions is termed as the gemistocytic astrocyte, wherein due to increased production of GFAP, they acquire abundant homogeneous-looking eosinophilic cytoplasm and eccentrically placed nuclei (Vinters and Kleinschmidt-DeMasters 2015). Apart from morphologic heterogeneity, reactive astrocytes also display genomic heterogeneity in response to specific stimuli. Genomic profiling of mice reactive astrocytes treated with systemic injection of lipopolysaccharide (LPS) (to induce neuroinflammation) or middle cerebral artery occlusion (to induce cerebral ischemia) revealed two main subtypes which were either detrimental or beneficial (Zamanian et al. 2021). These were termed as "A1" and "A2" (in analogy to the "M1"/"M2" macrophage nomenclature) (Liddelow and Barres 2017).

4 A1 Subtype

- A1 constitutes the neuroinflammatory subtype (seen following injection of LPS) characterized by upregulation the initial part of the classical complement cascade (C1r, C1s, C3, and C4) (Liddelow and Barres 2017).
- Microglia induce A1s by releasing three cytokines: interleukin 1 alpha (IL1a), tumor necrosis factor alpha (TNFa), and the complement component subunit 1q (C1q) (Liddelow et al. 2017).
- This subtype shows loss of normal functions of astrocytes (decreased ability to induce synapse formation and function, a loss of ability to phagocytose synapses, and a loss of ability to promote neuronal survival and growth) and toxic gain of new function (secretion of a yet-to-be-identified neurotoxin that induces apoptosis of neurons and oligodendrocytes). They are also responsible for the death of axotomized CNS neurons (Liddelow and Barres 2017). In addition, aged astrocytes display reactive phenotype of neuroinflammatory A1-like reactive phenotype which could contribute to the cognitive decline with aging (Clarke et al. 2018).
- Genes induced in A1 astrocytes include C3, H2-T23, SERPING1, H2-D1, GGTA1, IIGP1, GPP2, FBTLN5, PSMBB8 (Miller 2018; Zamanian et al. 2021).
- A1 neuroinflammatory reactive astrocytes might be induced by NF-κB (nuclear factor kappa light-chain enhancer of activated B cells) signaling which is activated in response to infectious agent (Liddelow and Barres 2017; Liu et al. 2017).

5 A2 Subtype

- A2 constitutes ischemia induced subtype (seen following middle cerebral artery occlusion) characterized by upregulation of neurotrophic factors which promote survival and growth of neurons, along with thrombospondins, which promote

synapse repair. This subtype is exemplified as being helpful aiding tissue repair (Liddelow and Barres 2017).

- Genes induced in A2 astrocytes include CLCF1, TGM1, PTX3, S100A10, SPHK1, CD109, PTGS2, EMP1, SLC10A6, TM4SF1, B3GNT5, CD14, and STAT3 (Miller 2018; Zamanian et al. 2021). S100A10 is identified as specific marker of A2 astrocyte which is essential for cell proliferation, membrane repair, and inhibition of cell apoptosis (Liddelow et al. 2017).
- A2 astrocytes also promote the expression of anti-inflammatory cytokine TGFβ, which participates in synaptogenesis and plays a neuroprotective role (Xu et al. 2018).

Although A1 reactive astrocytes are deemed neurotoxic, they may be essential to control the initial infection by release of molecules such as pro-inflammatory cytokines, chemokines, and intracellular kinases. Perhaps at a specific time point after control of infection, these may serve as a threat to the microenvironment. Intervention at this stage by blocking the upstream molecules or the downstream targets of A1 reactive astrocytes may constitute a valuable therapeutic option. Studies suggest that A1 astrocytes have the potential to transform to A2 astrocytes and even to naïve astrocytes (Li et al. 2019). Conversion of A1 to A2 may further aid in tissue repair.

The role of astrocytic subtypes in the management of chronic postsurgical pain has been extensively studied in rat models. Microglia induce the transformation of A1/A2 reactive astrocytes via the CXCR7/PI3K/Akt pathway. Li et al. demonstrated that minocycline (a microglial inhibitor) and AMD3100 (a CXCR7 agonist) reverted the A1/A2 ratio of reactive astrocytes and relieved the mechanical allodynia via the PI3K/Akt pathway (Li et al. 2020). Intrathecal injection of the astrocyte inhibitors, such as valerine, fluorocitrate, and l-1-amino-hexanedioic acid, has effectively reversed mechanical allodynia; however, specific inhibition of A1 reactive astrocytes could represent a potential therapeutic target that is more accurate and has fewer side effects (Li et al. 2019). Selectively modulating the A1 Astrocytes by nanovector loaded with rolipram, an anti-inflammatory drug in spinal cord injury model, limited the inflammatory response by reducing iNOS and Lcn2, which in turn reversed the toxic effect of proinflammatory astrocytes on motor neurons in vitro (Vismara et al. 2020). There is increasing awareness of these astrocytic subtypes in the fields of neurodegeneration, aging, chronic pain, neurotrauma, and stroke. However, there is paucity of literature related to these reactive astrocytic subtypes and their role in various CNS infections. The ratio of these subtypes may vary not only from one infectious agent to another but also based on the immune status of the individual. Available information in literature related to astrocyte physiology and pathology in various CNS infections is briefly reviewed below.

6 Pathobiology of Astrocytes in Various CNS Infections

6.1 Viral Infections (Figs. 1, 2, 3, 4, 5, and 6)

Herpesvirus Infections

Herpes simplex virus (HSV) 1 causes encephalitis in immunocompetent healthy individuals, in contrast to cytomegalovirus (CMV) encephalitis seen in severely immunocompromised patients. These opposing forms of herpes viral infections reflects the dual nature of immune response to infection (Lokensgard et al. 2002). Cultures of astrocytes and neurons infected with HSV show productive viral infection with cytopathic effects resulting in cell death, in contrast to microglial cell cultures which show only limited replication with death of these microglial cells mediated through apoptotic pathways. Neither of these fully permissive cell types (astrocytes and neurons) produce any cytokines or chemokines in response to HSV

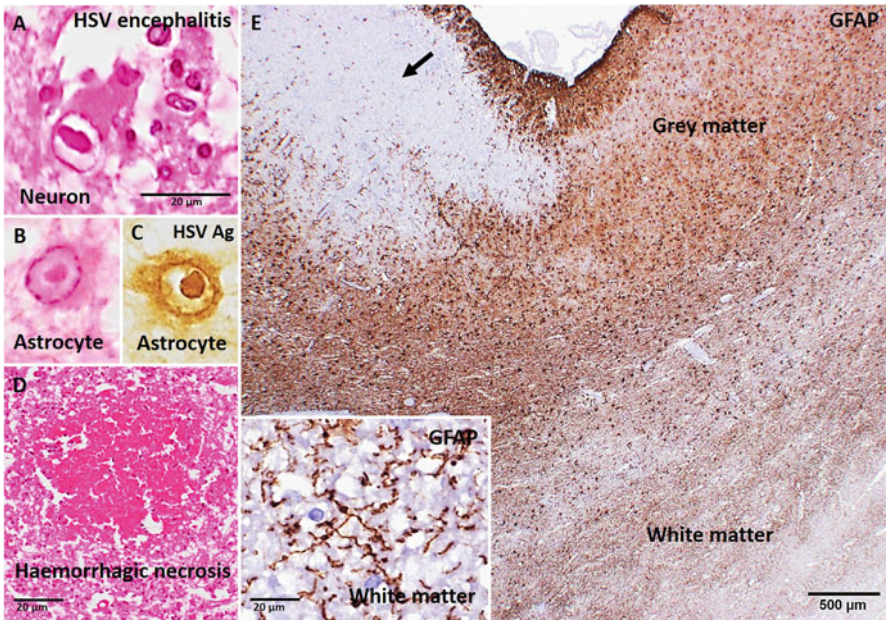

Fig. 1 HSV encephalitis is characterized by Cowdry-type A intranuclear eosinophilic inclusions, with peripheral halo and margination of chromatin towards the periphery. These inclusions are seen within neurons (**a**) and glia, particularly the astrocytes (**b**). IHC for HSV antigen is used to confirm the diagnosis which stains the viral particles within the inclusion and in the marginated rim of chromatin (**c**). Affected areas in HSV encephalitis include the medial temporal lobes, orbitofrontal cortex, and limbic areas which show hemorrhagic necrosis (**d**). In addition, features common to any viral encephalitis such as neuronophagia (**a**), perivascular lymphocytic infiltrate, and microglial nodules are seen (not shown in the figure). IHC for GFAP shows reactive astrogliosis in the subpial, cortical, subcortical regions (**e**). The white matter showed dystrophic changes in the astrocytic processes with beading and fragmentation (inset, **e**). Areas of hemorrhagic necrosis were unstained indicating complete destruction of astrocytes in these regions (arrow, **e**)

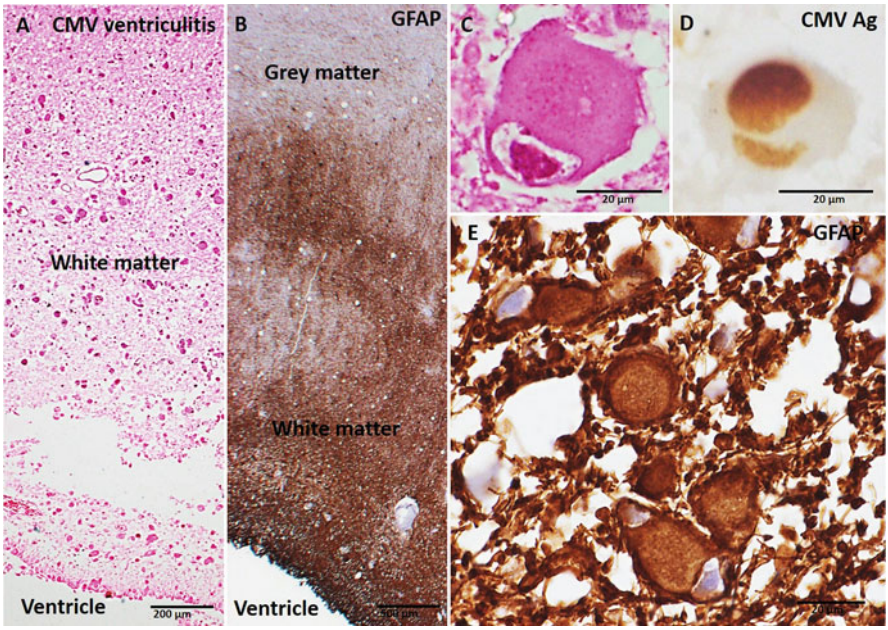

Fig. 2 CMV encephalitis presents most commonly as ventriculitis in immunocompromised individuals. The periventricular area shows a thick band of necrosis and denudation of ependymal lining of the ventricles (a). Several of the cells (macrophages, ependymal cells, endothelial cells, glia) within this area show cytomegaly with prominent intranuclear basophilic Cowdry-type A inclusions (owl eye inclusions) (**c**). Viral particles can be demonstrated by CMV antigen which stains not only the intranuclear inclusions but also intracytoplasmic inclusions within the cytomegalic cells (**d**). IHC for GFAP shows corresponding dense gliosis within the periventricular white matter and minimal reactive glial cells in gray matter (**b**). It also stains several of these inclusion bearing cells (**e**)

infection. However, microglia respond to nonpermissive HSV infection by producing TNF-α, IL-1β, IP-10, and RANTES together with smaller amounts of IL-6, IL-8, and MIP-1a (Lokensgard et al. 2001). Primary murine cortical cultures have shown activated microglia secrete TNF-α to promote the A1 profile of astrocytes with upregulation in the A1 marker CXCL10. However, at the same time, increase in the A2-marker Cox2 was also demonstrated. Possible explanation for this was an unconventional astrocyte-activation, a mixed cell population of A1 and A2 cells or astrocytes displaying a continuum between A1 and A2 subtype. Fibroblast growth factors (FGFs) secreted from HSV-1-infected CNS cells may be anti-inflammatory via astrocytic FGFR activation. FGFs may mediate the important A1 to A2 shift thereby promoting repair over removal of damaged cells (Hensel et al. 2019).

CMV-infected astrocyte cell cultures show permissive infection with cytopathic effects and cell death, but not the mature neurons and microglial cells reflecting non permissive nature within these cell types. CMV-infected astrocytes release substantial amounts of the chemokines MCP-1, MIP-1a, and IL-8. These chemokines then

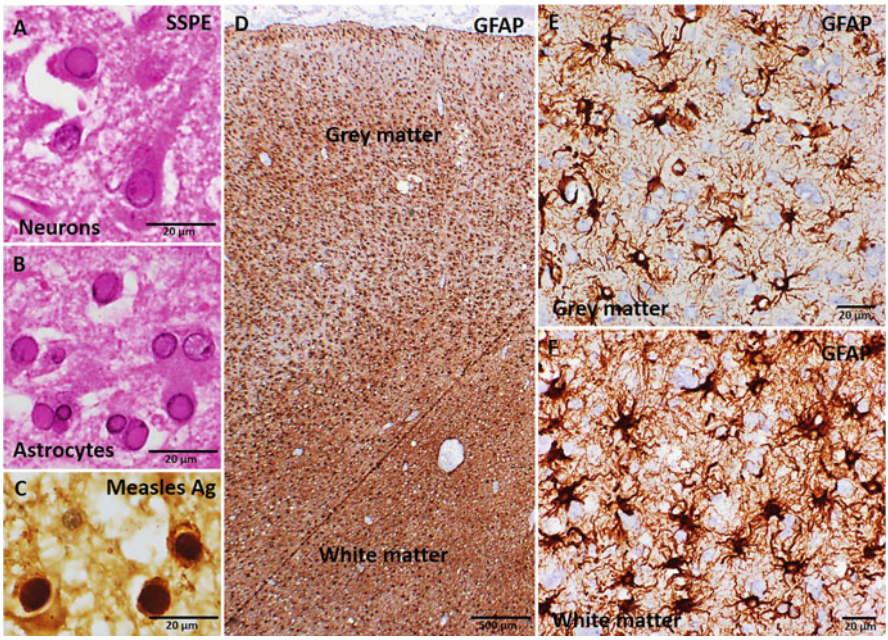

Fig. 3 Subacute sclerosing panencephalitis (SSPE) occurs years after an initial infection by measles virus in immunocompetent children. It is characterized by intranuclear Cowdry-type A inclusions within neurons (**a**) and glial cells, including astrocytes (**b**) and oligodendrocytes. Inclusions containing the viral particles are demonstrated by IHC to measles antigen (**c**). GFAP shows marked reactive isomorphic astrogliosis in both the grey and white matter (**d**, **e**, **f**) which has coined the term sclerosing for this form of encephalitis

recruit other immune cells to local sites of infection. CMV stimulated microglia produce the antiviral cytokine TNF-a plus IL-6, MCP-1, IL-8, RANTES, IP-10, and MIP-1a. Even though astrocytes are not capable of defending themselves against CMV infection, they have the capacity to produce chemokines that may recruit cells such as microglia that have antiviral properties to the site of infection (Lokensgard et al. 1999). Chemokines initiate a cascade of neuroimmune responses that result in defense of and damage to the brain, which involves both activated glia and infiltrating T cells.

Astrocytes are the main target and reservoir of human herpes virus 6 (HHV6) in the CNS, which can cause encephalitis in both healthy and immunosuppressed patients. There are two variants of this virus: HHV-6A and HHV-6B. In cell culture study utilizing human progenitor-derived astrocytes demonstrated that HHV-6A and HHV-6B have differential tropisms and patterns of infection, where HHV-6A results in a productive lytic infection and HHV-6B is associated with a nonproductive infection, thus responsible for the specific infection patterns in glial cells in vivo (Donati et al. 2005). Persistent viral infection of the CNS with subsequent reactivations has been documented in some patients after stem cell transplantation (Wainwright et al. 2001). HHV-6B-infected astrocytes are defective in maintaining

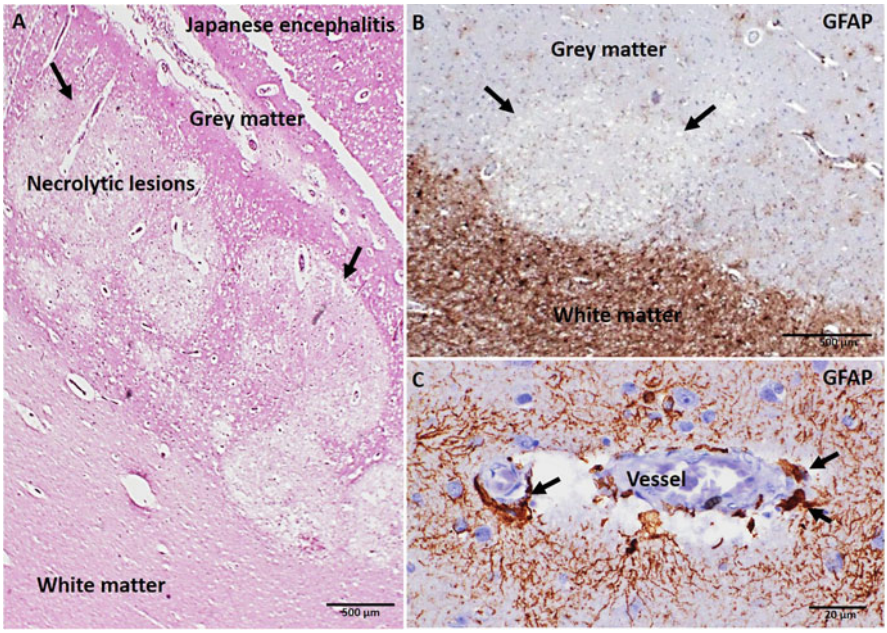

Fig. 4 Japanese encephalitis is a polioencephalitis affecting the gray matter of the cortex and deep nuclear areas (thalamus). The cortical ribbon shows circumscribed necrolytic lesions which appear pale acellular on H & E (arrow, **a**) and are devoid of astrocytes as shown by GFAP (arrow, **b**). Perivascular astrocytes are prominent on GFAP (**c**) with hypertrophic processes surrounding the endothelial cells (arrow, **c**). This feature could possibly be related to the route of entry of this virus into the CNS which infects the endothelial cells and then crosses the BBB

the homeostatic balance due to altered glutamate uptake with subsequent neuronal dysfunction (Fotheringham et al. 2008).

Flavivirus Infections

Tick-borne encephalitis virus (TBEV) is one of the neurotropic flaviviruses causing meningitis, meningoencephalitis, and myelitis in endemic regions of Europe and Asia. Astrocytes are a potential mediator of brain infection and a reservoir in TBEV. TBEV successfully replicates in astrocytes, reaching a higher viral load but is relatively resistant to cell death (Potokar et al. 2014). This induces several morphologic and physiologic changes in astrocytes within the cytoskeleton particularly in actin and tubulin cytoskeleton polymerization with significant effect on TBEV-laden vesicle trafficking (Potokar et al. 2007). Modalities that modify this vesicle traffic within the TBEV-infected astrocytes may act as a potential therapeutic intervention (Potokar et al. 2019). TBEV also triggers astrocyte activation evident by increased GFAP along with release of inflammatory cytokines and chemokines. Infection of astrocytes alters the blood-brain barrier (BBB) permeability via overexpression of matrix metalloproteinase-9 and affects the functioning of neighboring cells including neurons (Palus et al. 2014). The infected astrocytes also act protectively by

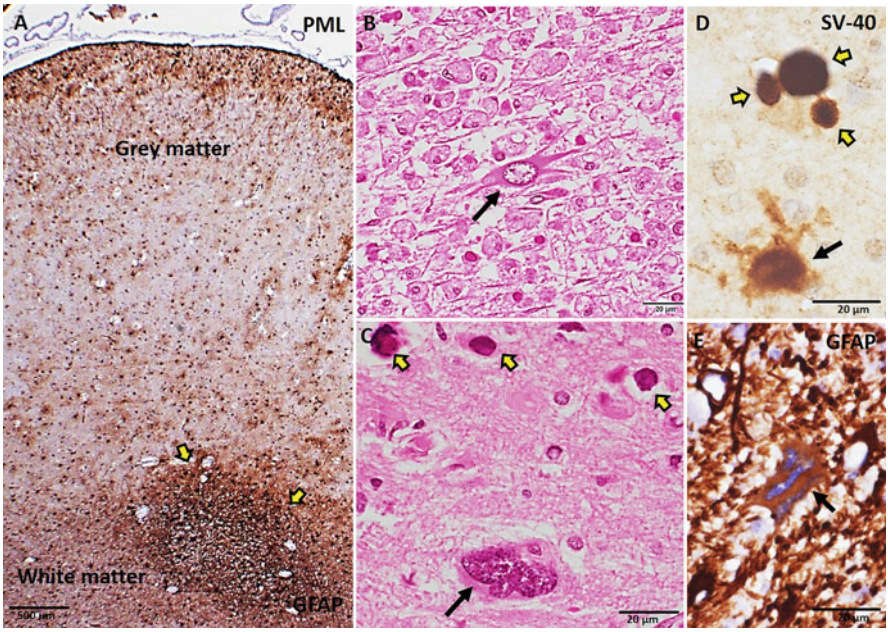

Fig. 5 Progressive multifocal leukoencephalopathy (PML) caused by JC virus productively replicates in the oligodendroglial cells with their subsequent death leading to demyelination within the white matter. The areas of demyelination show pallor and infiltration by sheets of foamy macrophages (**b**). The surviving oligodendrocytes show enlarged nucleus with intranuclear inclusion which is basophilic and completely occupies the nucleus (yellow arrow, **c**). The astrocytes which are nonproductively infected undergo morphological changes with hypertrophied giant astrocytes (black arrow, **b**) showing multinucleation, bizarre nuclei, and coarse vesicular chromatin (black arrow, **c**). IHC to SV-40 antigen demonstrates the viral particles within the oligodendroglial nuclei (yellow arrow, **d**) and in the astrocytes (black arrow, **d**) wherein they take a granular positivity representing the nonpermissive infection of astrocyte. GFAP shows dense reactive gliosis in the demyelinating areas within the white matter (yellow arrow, **a**) and mild subpial gliosis in grey matter (**a**). Bizarre astrocytes are stained with GFAP (**e**)

upregulation of type 1 IFNs which restricts the spread and replication of virus (Lindqvist et al. 2016).

Japanese encephalitis virus (JEV) transmitted by mosquitoes is one of the common causes of viral encephalitis in Asia-Pacific region and northern Oceania. Nearly half of infected patients are left with permanent neuropsychiatric sequalae. JEV infects the brain endothelial cells with subsequent crossing of the BBB. Details of how the virus enters the astrocytes are not defined (Potokar et al. 2019). It replicates and triggers morphologic changes within astrocytes, with production of inflammatory cytokines and chemokines which lead to alteration of the BBB permeability (Li et al. 2015). Furthermore, chemokines such as RANTES released from astrocytes might play a role in the recruitment of immune cells (Chen et al. 2011). Production of type I IFN by astrocytes in response to JEV limits the spread of the virus and

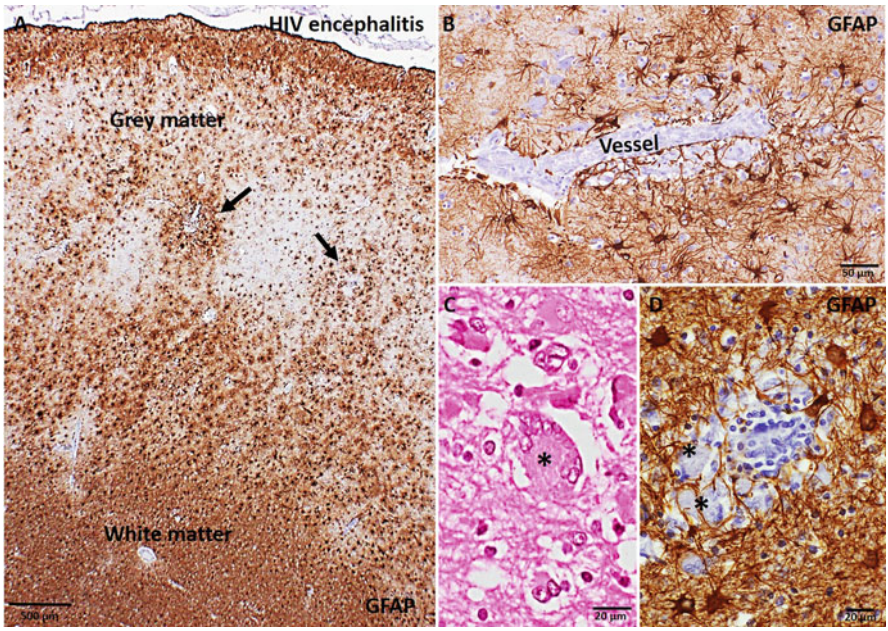

Fig. 6 HIV encephalitis associated with neurocognitive decline is characterized by the presence of microglial nodules composed of activated macrophages which have fused to form multinucleated giant cells (asterix, **c**). These microglial nodules are enveloped by reactive stellate astrocytes as highlighted by GFAP (**d**). The multinucleated giant cells are negative for GFAP (asterix, **d**) as they belong to monocyte/macrophage lineage. IHC for GFAP shows moderate amount of reactive astrogliosis involving both the gray matter and white matter (**a**), with distinct perivascular aggregation of astrocytes (arrow **a**, **b**)

prevents virus-induced killing of the neighboring astrocytes and neurons (Lindqvist et al. 2016).

Another arboviral infection caused by neuroinvasive stains of West Nile Encephalitis Virus (WNV) prevalent in Africa, Europe, and North America, is associated with severe long-term neurological consequences. The virus enters the brain invading across the BBB and infects the astrocytes prior to infection of neurons (Potokar et al. 2019). Although both neurons and astrocytes support productive WNV infection, the virus replicates at a higher and faster rate in the astrocytes. Viral growth though poorly permissive in microglial cells, releases robust amounts of proinflammatory cytokines and chemokines (Cheeran et al. 2005). WNV infection in the primary cultures of mouse neurons were rapidly progressive and destructive; on the other hand, infected astrocytes show a permissive, persistent infection playing a role in the maintenance of the virus in CNS (Diniz et al. 2006). Astrocytes regulate the spread of WNV within the CNS and therefore are an attractive target for ameliorating WNV-induced neuropathology. The severity WNV infection is strain dependent, ranging from avirulent to highly neuropathogenic and is interferon-independent, related to the replication dynamics within astrocytes and

astrocyte-specific restriction of WNV particle production through furin-like proteases (Hussmann et al. 2013, 2014). Astrocytic infection by WNV results in induction of neuroinflammatory genes particularly CXCL10 which can act as a neurotoxin inducing apoptotic cell death by increasing intracellular Ca2+ levels. CXCL10 also acts as a neuroprotectant by recruiting immune cells into the CNS to clear infection. WNV also exerts its effect on endoplasmic reticulum stress response by downregulation of the protective astrocyte-specific endoplasmic reticulum related transcription factor gene, rendering it no longer protective (van Marle et al. 2007).

Zika virus (ZIKV) outbreaks in South America and the Caribbean have unfolded this arbovirus to be associated with vide range of neurologic symptoms, particularly affecting the fetus with congenital Zika syndrome which includes a myriad of abnormalities, including microcephaly, lissencephaly, hydrocephalus, arthrogryposis, and parenchymal calcifications (Sejvar 2018). Fetal autopsy of ZIKV-infected fetus at 32 weeks has shown diffuse astrogliosis with focal astrocytic outburst into the subarachnoid space, mostly on the convexity of the cerebral hemispheres (Mlakar et al. 2016). Astrocytes and microglia are the targets of entry for ZIKV via AXL, a group of tyrosine kinase receptors involved in the clearance of apoptotic cells and regulation of innate immunity. AXL kinase activity downmodulates interferon signaling and facilitates infection. Inhibiting AXL function may represent a potential target for future antiviral therapies (Meertens et al. 2017). ZIKV shows tropism for radial glia and astrocytes which are more susceptible to infection than neurons, a pattern that correlates with expression of a putative viral entry receptor, AXL (Retallack et al. 2016). ZIKV-infected astrocytes manifest programmed cell death with a progressive cytopathic effect and massive vacuolization leading to paraptosis, a caspase-independent, non-apoptotic form of cell death associated with the formation of large cytoplasmic vacuoles (Monel et al. 2017). ZIKV infection in human fetal astrocytes shows a considerable increase in extracellular vesicles which are the Trojan horses of viral infection (Altan-Bonnet 2016; Huang et al. 2018). Infected astrocytes are associated with an increased expression of a relatively narrow spectrum of proinflammatory cytokines and chemokines compared to other flaviviruses such as TBEV. This limited activation of the immune response may be associated with high infection rates and high titer virus production (Stefanik et al. 2018). Zika virus infection disrupts neurovascular development, leads to progressive astrogliosis, and altered permeability of the BBB, ultimately resulting in postnatal microcephaly with significant brain damage (Shao et al. 2016).

Human Immunodeficiency Virus (HIV)

HIV enters the CNS during seroconversion utilizing the Trojan horse mechanism via the infected macrophages. Microglia and macrophages are the principal cell types to be productively infected by HIV and produce many cytokines and chemokines. Astrocytes remain nonproductively infected. Several studies have demonstrated that efficient HIV-1 replication is blocked in astrocytes at different steps of the virus life cycle, including virus entry, reverse transcription, nucleocytoplasmic HIV-1 RNA transport, translation of viral RNA, and maturation of progeny virions (Gorry et al.

2003). As astrocytes are devoid of CD4 and chemokine receptors which are key to infection of immune cells, there are some alternative receptors on astrocytes that play a role as potential HIV-1 receptors on astrocytes that include proteins with molecular masses of 260 kDa and 65 kDa, which bind gp120, galactosyl ceramide, and human mannose receptor. The 65 kDa protein and the human mannose receptor could mediate endocytosis of HIV-1 into astrocytes (Kramer-Hämmerle et al. 2005). Astrocytes act as reservoirs for HIV. The malfunction of Rev in astrocytes hardly allows the production of progenitor virus, but high levels of the regulatory proteins Nef, Rev, and Tat are detected (Brack-Werner 1999). Studies have also suggested that under the appropriate environmental milieu, astrocytes can support productive HIV replication. IFN-γ induces HIV replication in astrocytes highlighting that the environmental milieu is critical in regulating the permissiveness of astrocytes to HIV infection. IFN-γ diminishes the β-catenin signaling in astrocytes, a pathway that is a potent inhibitor of HIV replication. The Wnt/β-catenin pathway also plays an important role in axonal remodeling, regulation of synaptic connectivity and regulation of hippocampal neurogenesis in the adult brain. Modulation of this β-catenin pathway can impact viral infection and favor neurogenesis and neuroprotection opposed to the toxic insult (Li et al. 2011).

HIV infection in the CNS promotes neuronal injury and/or neurotoxicity through the release of soluble factors produced by infected macrophages/microglia and astroglial cells. HIV encephalitis is associated with a significant increase in MHC class II on activated microglia and astrocytes, and it is considered the best neuropathologic correlate of cognitive impairment (Persidsky and Poluektova 2006). A postmortem study on brains of HIV+ patients with neurocognitive impairment revealed that astrocyte infection by the virus was extensive in subjects with HIV-associated dementia (HAD) and correlated with the severity of neuropathological changes and proximity to perivascular macrophages (Churchill et al. 2009). Neuronal excitotoxicity is prevented by astrocytes via the clearance of extracellular glutamate by a family of transporter proteins, excitatory amino acid transporters (EAAT). Inflammatory mediators, in particular the cytokine TNF-α, and HIV viral proteins reduce the expression of EAAT on astrocytes resulting in impaired glutamate clearance. Furthermore, the trophic action of astrocyte to provide the amino acid glutamine via their expression of glutamine synthetase is also hampered in HIV. An interesting concept is that the activated microglia and brain macrophages partly compensate for the inhibited astrocytic function as they express the transporters and enzymes of the glutamate cycle thus exhibiting neuroprotective properties (Gras et al. 2006).

In our study, association of HIV with opportunistic infections resulted in muted glial and microglial response. The reactive astrocytic response surrounding parenchymal pseudocystic cryptococcal lesions was less in HIV-positive cases, compared to HIV negative cases. The reactive astrocytes and microglia following opportunistic infection developed dystrophic changes, heralding senescence. Both astroglial and microglial cells also expressed caspase-3, a pro-apoptotic marker. The death of astrocytes with loss of support mechanisms compromise the surrounding neurons

which could lead to neurodegeneration following chronic inflammation (Tripathi et al. 2014).

Progressive Multifocal Leukoencephalopathy (PML)

PML caused by JC polyomavirus causes a fatal demyelinating disease following reactivation in immunosuppressed individuals. Demyelination is attributed to the productive infection and lytic destruction of the myelinating oligodendrocyte. The other cell type affected is the astrocyte wherein it establishes a latent infection due to nonpermissive infection. This leads to morphological abnormality and cytopathic change in the form of hypertrophied giant astrocyte with multinucleation and bizarre nuclei to the extent that it mimics a glial neoplasm (Love et al. 2015). JC polyomavirus in normal human astrocytes has delayed progression of the infectious cycle with significant reduction of the late viral gene product VP1 (Wilczek et al. 2020). Ultrastructural studies have demonstrated viral adsorption, penetration, and intracellular transport in protoplasmic and fibrous astrocytes. The early steps of the viral infection were more frequently found in the cytoplasm of the astrocytes than virus assembly within their nucleus, suggesting that the early steps of viral replication are not always followed by virus production. It was also noted that infection was more intense, when giant astrocytes were absent than when giant cells were formed. Ultrastructural evidence of viral particles has been described within the astrocytic nuclei, however none in giant astrocyte (Mazlo and Tariska 1982). JCV can infect granule cell neurons of the cerebellum, causing JCV granule cell neuronopathy and cortical pyramidal neurons in JCV encephalopathy. JCV has also been demonstrated within the hippocampal neurons and its afferent and efferent white matter tracts leading to seizures and cognitive dysfunction. The infected neurons in this study expressed JCV T antigen only, suggesting an abortive/restrictive infection, whereas the glial cells expressed either JCV regulatory T Antigen or JCV VP1 capsid protein (Wuthrich et al. 2016).

6.2 Bacterial Infections (Figs. 7 and 8)

Bacterial Meningitis

Meningitis involves inflammation of the leptomeninges which if not treated and can extend beyond the subarachnoid space, evolving into meningoencephalitis or cerebritis leading to severe parenchymal inflammation injury and necrosis. The primary anatomical barrier preventing spread of inflammation into the parenchyma is the glia limitans, at the pial-subarachnoid interface by plump foot processes of reactive astrocytes, fibromeningeal cells, and an overlying basement membrane. This shares homology with glial scar formation. Breach of the glia limitans is attributed to the various toxic molecules released by the neutrophils or as a result of occlusion and thrombosis of meningeal vessels leading to frank necrosis (Sofroniew and Vinters 2010).

Staphylococcus aureus is one of the major etiological agents of bacterial meningitis and pyogenic abscess. Astrocytes are capable of recognizing this Gram-positive

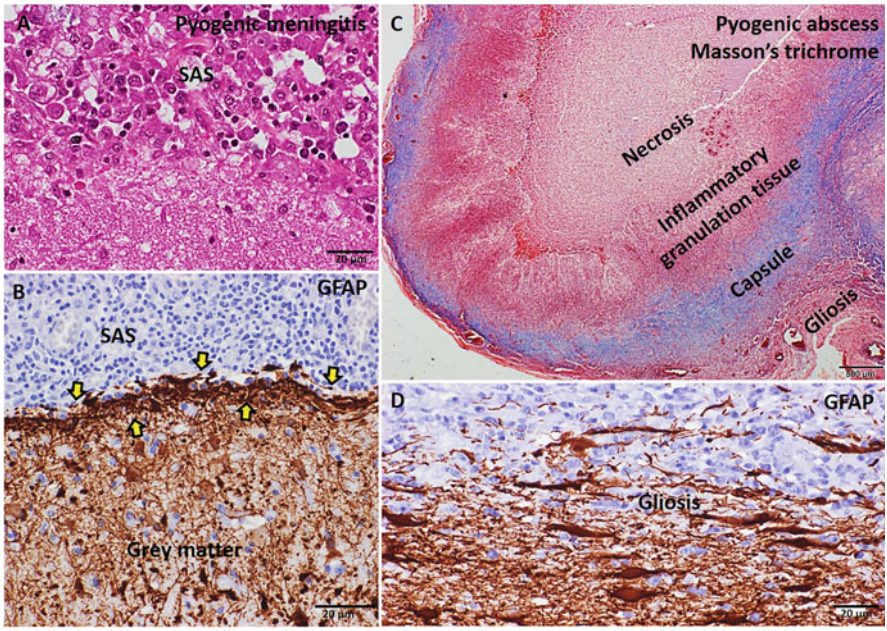

Fig. 7 Pyogenic meningitis showing subarachnoid space (SAS) filled with inflammatory infiltrate (**a**). The glia limitans at the pial-subarachnoid interface is prominent on GFAP (arrow, **b**) which is formed by the plump foot processes of reactive astrocytes limiting the spread of infection into the parenchyma. Subpial cortical reactive gliosis is also evident (**b**). Pyogenic abscess is composed of four layers from within outwards comprising liquefactive necrosis, inflammatory granulation tissue, fibrous capsule, and gliosis in the adjacent parenchyma (Masson's trichrome stain, **c**). The capsule once formed helps in containing and localizing the infective process and limiting its spread to surrounding parenchyma. The capsule is composed of collagen fibers laid down by proliferating fibroblasts which are intermingled with glial processes which strengthen the capsule. The surrounding neuroparenchyma is edematous with persistent activation of astrocytes as highlighted by GFAP (**d**) and microglial cells

bacterium and its cell wall product peptidoglycan by the Toll-like receptor 2 (TLR2) and respond by producing numerous proinflammatory mediators including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-1β (MIP-1β), MIP-2, and monocyte chemoattractant protein (MCP-1) (Esen et al. 2004). Astrocytes form an extensive syncytium via the gap junction channels composed of protein subunits called connexins. Staphylococcus aureus and peptidoglycan has shown to cause time-dependent decrease in expression of connexins resulting in decreased coupling between cells surrounding infection. Under physiological conditions, the astrocyte syncytium is crucial for neuronal homeostasis but, the same in pathological conditions, may lead to the propagation of apoptotic and/or necrotic signals at distant sites within injured tissue leading to extended neuronal injury. Inhibition of gap junction channels in response to injury could represent a protective defense mechanism (Esen et al. 2007).

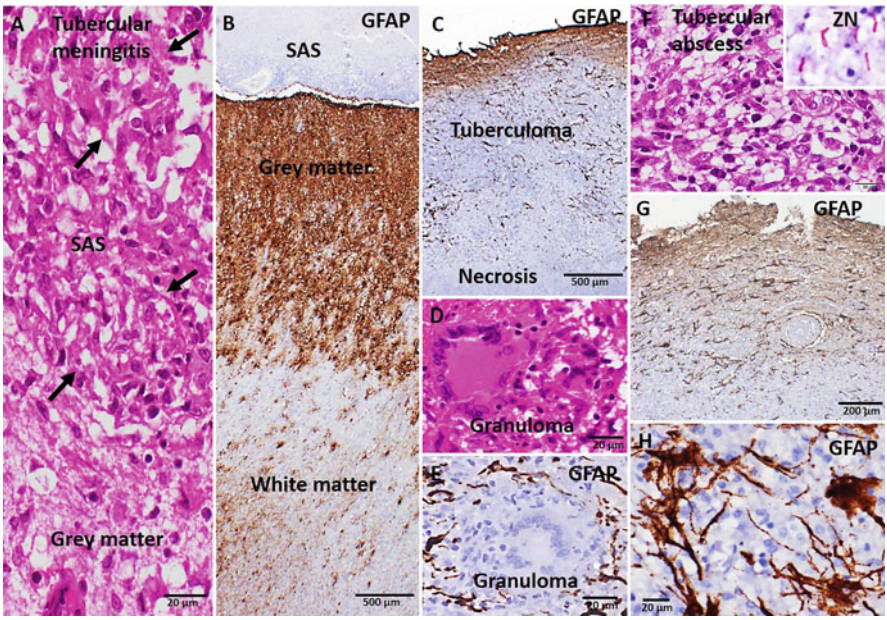

Fig. 8 Tubercular meningitis showing chronic granulomatous inflammation in the subarachnoid space (SAS) with well-formed epithelioid granulomas (arrow, **a**). GFAP shows prominent pial glial limitans which is the first barrier to restrict parenchymal spread. The cortex shows decreasing gradient of glial cell density in the lower cortical layers, suggesting a spread of transducing signals from the inflammatory focus along the surface. Tuberculoma is a space occupying lesion which is defined histopathologically by the central area of necrosis surrounded by well-formed epithelioid granulomas with multinucleated Langhans type of giant cells (**d**) along with dense cuff of chronic inflammatory cells. GFAP stains the gliosis surrounding the tuberculoma and glial process within the capsule enclosing the lesion (**c**). Individual granulomas are enclosed by thin glial processes (**e**). Tubercular abscess is seen in immunocompromised individuals which histologically mimics a pyogenic abscess showing mixed inflammation (**f**), without any epithelioid granulomas. However, Ziehl-Neelsen stain (ZN stain) shows numerous acid-fast bacilli within the lesion (inset, **f**). The immunocompromised state precludes the development of granulomas. GFAP stains reactive astrocytes within and the periphery of lesion (**g**). However, the glial cells are dystrophic with abnormal shapes and show hypertrophic glial processes (**f**) probably reflecting the altered immune response

Meningitis, the most common acute manifestation of Lyme neuroborreliosis is caused by gram negative spirochete, Borrelia burgdorferi. The role of astrocytes in the immune response to B. burgdorferi in the CNS has been evidenced by human CSF samples in which GFAP was increased early in course of CNS disease and decreased following antibiotic treatment (Dotevall et al. 1996). Rhesus monkeys infected with B. burgdorferi showed evidence of astrocyte proliferation and apoptosis, which was hypothesized to be caused by spirochetal lipoproteins (Ramesh et al. 2003). Human astrocytes expressed MMP-9 when incubated with B. burgdorferi contributing to the breakdown of BBB (Perides et al. 1999). They also play an important role in recruiting inflammatory cells to the CNS following exposure to this

spirochete by production of chemokines including IL-6, TNF-α, IL-8, CXCL-1, and CXCL-10 (Brissette et al. 2013).

Tubercular Meningitis (TBM)

Tubercle bacillus preferentially infects human microglia rather than astrocytes. Microglia are productively infected with Mycobacterium tuberculosis and are the principal effectors initiating, orchestrating, and modulating the tuberculous immune response by secretion of various cytokines (TNF-a, IL-1b, and IL-6) and chemokines (CCL2, CCL5, CXCL8, and CXCL10), inducing the differentiation of CD4+ T-lymphocytes which ultimately eliminate or contain the infection. Astrocytes challenged with tubercle bacilli have a much narrower cytokine-chemokine response with detection of moderate amounts of only a single chemokine, CXCL10 (Rock et al. 2005; Spanos et al. 2015). Astrocytes contribute to a matrix degrading environment within the CNS and subsequent morbidity and mortality (Harris et al. 2007). Mycobacterium tuberculosis stimulation activates astrocytes leading to upregulation of MMP secretion, with consequent disruption of the BBB, breakdown of type IV collagen, and the downregulation of the tight junction proteins ZO-1, claudin-5, and occludin. Impaired tight junctions together with increased surface expression of endothelial adhesion molecules (e.g., ICAM-1, VCAM-1) result in leukocyte influx into the CNS, causing host immunopathology which is responsible for much of the morbidity and mortality in CNS tuberculosis (Brilha et al. 2017).

In a detailed morphologic and morphometric study of TBM cases diagnosed and archived at our center, the alterations in microglia and astrocytes were evaluated. The activated microglia and astrocytes displayed hypertrophy and hyperplasia, aggregating in proximity to the meningeal exudates. A decreasing gradient of glial cell density, GFAP and S-100β expression, was evident in the lower cortical layers, suggesting a spread of transducing signals from the inflammatory focus along the surface. Dense glia limitans with gliosis indicated the efforts to restrict the spread of inflammatory process into the adjacent parenchyma by the pia-glial limitans. We also observed a difference in the morphology of astrocytes in cases of TBM with and without associated HIV infection. In the HIV-negative TBM cases, the astrocytes surrounded the parenchymal granulomas and had small rounded hypertrophied cell bodies with intense GFAP labelling and had short stout processes, resulting in a lower area fraction. In the HIV-positive cases, the astrocytic processes were long, slender, and more conspicuous, resulting in higher area fraction (Tripathi et al. 2014).

6.3 Fungal Infections (Figs. 9 and 10)

Cryptococcal Meningoencephalitis

The neurotropic fungi Cryptococcus neoformans is an encapsulated yeast causing life-threatening meningoencephalitis in immunocompromised hosts with minimal inflammatory response and glial activation. The number of cryptococcal yeast inversely correlates with the number of inflammatory cells. This reveals inefficient

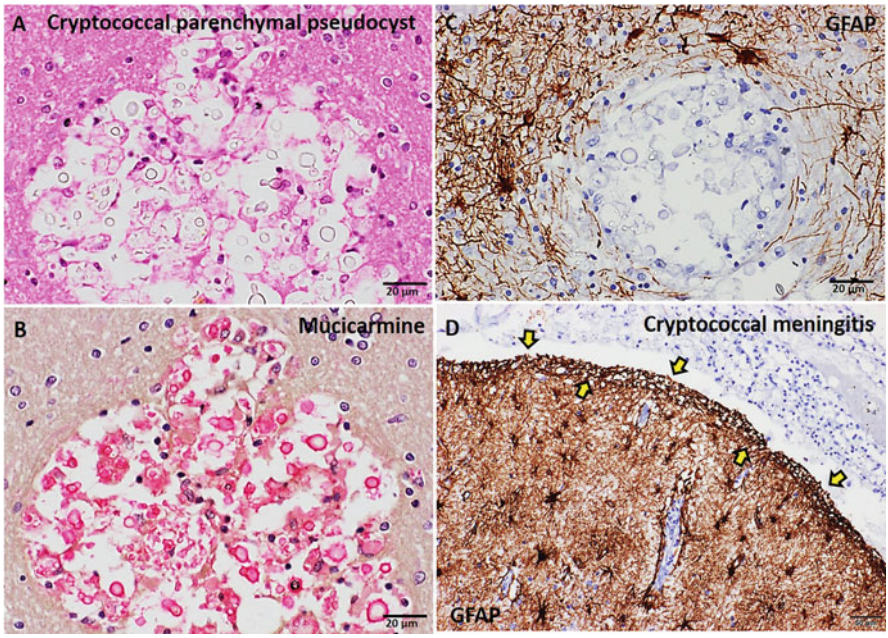

Fig. 9 Cryptococcal meningitis in immunocompromised individuals is characterized by pseudocystic lesions which represent dilated Virchow-Robin spaces containing aggregates of yeasts (**a**) giving a soap bubble appearance with neurotropism for the basal ganglia and substantia nigra. The capsule of cryptococci is stained with mucicarmine stain (**b**). GFAP stains stellate reactive astrocytes around the pseudocystic lesion (**c**). Prominent pial glia limitans (arrow, **d**) is seen restricting the infection in subarachnoid space along with subcortical reactive isomorphic gliosis (**d**)

signals for astrocyte activation. Study on primary culture of human fetal astrocytes activated with IL- β and INF-γ, reflected antifungal activity through NO mediated mechanism, and was reversed by inhibitors of NO synthase. Thus, therapeutic strategies directed at enhancing astrocyte activation may be helpful in the management of cryptococcosis (Lee et al. 1994). By virtue of their critical location, the perivascular and subpial astrocytes along with perivascular macrophages form an essential barrier to the spread to cryptococci. The principal effectors in cryptococcal meningoencephalitis are the macrophages and microglia, especially those in the perivascular and juxta vascular locations. The reactive astrocytes are limited to large destructive lesions and subpial regions (Lee et al. 1996).

In our study a differential reactive astrocytic response was observed encircling the cystic parenchymal cryptococcal pseudocysts, the reactive change being less when associated with HIV. The immunocompromised state of HIV probably influences the cytokine-induced astrocytic proliferation in cases of cryptococcal infection. Morphologically, in contrast to cases of TBM, in HIV-positive cryptococcal meningitis cases, the activated hypertrophic astrocytes revealed rounded cell bodies and retracted processes, whereas in HIV-negative cryptococcal meningitis cases, the astrocytes had prominent branching processes. In addition, focal areas showed

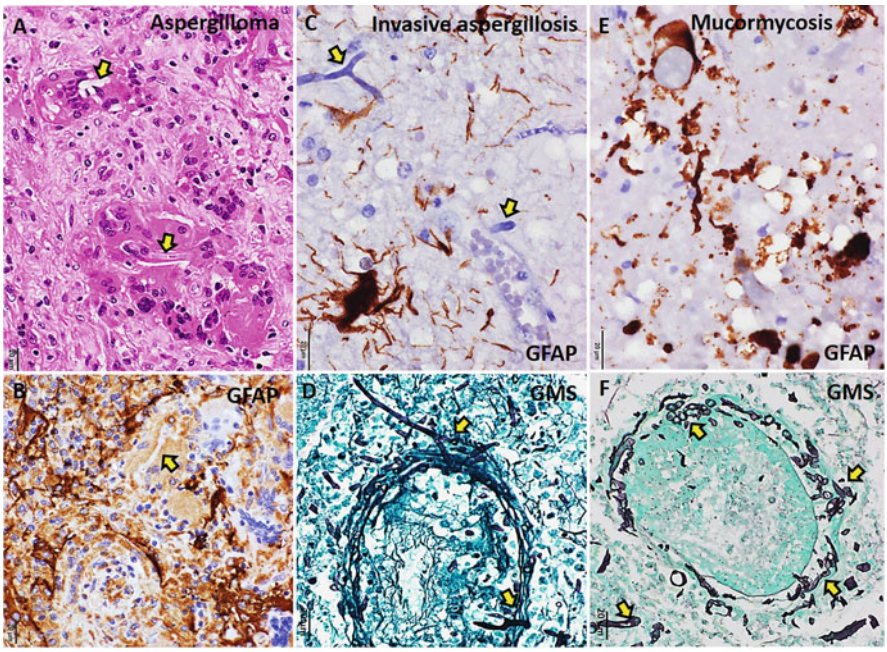

Fig. 10 Aspergilloma caused by fungal aspergillus species is a necrotizing granulomatous inflammation seen in immunocompetent individuals with several foreign body giant cells engulfing broken down fungal hyphae (arrow, **a**). GFAP stains reactive astrocytes surrounding the granuloma (**b**). Invasive aspergillosis seen in immunosuppressed state showing angioinvasion by slender septate fungal hyphae with branching at acute angles highlighted on Gomori methenamine-silver (GMS) stain (arrow, **d**). GFAP shows dystrophic hypertrophic astrocytes due to altered immune response (**c**). Mucormycosis caused by Mucorales species is a suppurative granulomatous inflammation seen in diabetic/immunosuppressed individuals characterized by wide spread angioinvasion and thrombosis by broad aseptate fungal hyphae which branch at right angles as highlighted on GMS stain (arrow, **f**). GFAP shows dystrophic astrocytes with breaking down and beaded astrocytic process probably due to altered immune response (**e**)

irregular breakdown and beading of astrocytic processes forming plaque-like zones, suggesting dystrophic change which was similar to cases of TBM. Many of the degenerating astrocytes along with subpial and perilesional astrocytes in both cryptococcal meningitis and TBM showed expression of caspase-3, a cellular apoptotic mediator (Tripathi et al. 2014).

Aspergillosis

Aspergillus species are a group of filamentous fungi which are found ubiquitously in soil and decaying vegetation. Aspergillus spreads to the CNS either hematogenously with a primary focus in the lung or locally following maxillary sinusitis. Infection of the CNS by Aspergillus species in immunocompetent individuals leads to the formation of aspergilloma, which is characterized on histology by well-formed epithelioid granulomas containing several foreign body giant cells with engulfed

broken-down hyphae. On the other hand, infection in immunosuppressed individuals leads to invasive aspergillosis, wherein the fungi typically invade vessel walls with luminal obliteration and subsequent necrosis. Aspergillus species produce and secrete factors which have toxic activity towards astrocytes, microglia, and neuronal cells (Speth et al. 2000). Gliotoxin is a mycotoxin produced by the Aspergillus strains involved in human aspergillosis (Lewis et al. 2005). Gliotoxin has shown to exhibit differential detrimental effect in the various cell types studied with highest susceptibility to the cultured astrocytes. Gliotoxin penetrates and impairs the integrity of the human blood-brain barrier by exerting adverse effect on brain endothelial cells. Immunocytochemistry analysis has shown an absence of obvious disruption of tight junction complexes in brain microvascular endothelial cell monolayers, whereas changes in β-catenin and F-actin were suggestive of a cytoskeleton remodeling. This suggests that impairment in cell-matrix rather than cell-cell interactions may act as a driving force in aspergillosis (Patel et al. 2018).

6.4 Parasitic Infections (Figs. 11, 12, and 13)

Toxoplasma Encephalitis

Toxoplasma encephalitis affects immunocompromised individuals with defective cell mediated immunity, due to reactivation of latent infection. Toxoplasma gondii is an obligate intracellular parasite which infects astrocytes, neurons, and microglia cells. Robust astrocyte activation is a hallmark of toxoplasma encephalitis (Drogemuller et al. 2008). The control of CNS toxoplasmosis is critically dependent on the local IFN-γ production by CD4 and CD8 T cells (Suzuki et al. 1989). In vitro studies of IFN- γ-activated astrocytes control the infection via the small inducible GTP binding protein (IGTP), which mediates disruption of parasitophorous vacuole, and deficient mice are unable to control the infection and succumb subsequently to necrotizing toxoplasma encephalitis (Halonen et al. 2001; Taylor et al. 2000). In response to T. gondii astrocytes produce a variety of cytokines and chemokines (IL-1a, IL-6, GM-CSF, MCP-1, IP-10, and PGE2) along with upregulation of MHC class I molecules (Fischer et al. 1997; Strack et al. 2002). IFN- γ-activated microglia releases the toxic metabolite nitric oxide (NO) which may cause neuronal injury and subsequent neurodegeneration. The PGE2 production by infected astrocytes is responsible for IL-10 secretion by microglia and downmodulating NO production, which consequently avoids neuronal damage. This cross talk between astrocytes and microglial cells is essential to maintain the CNS homeostasis during T. gondii infection (Rozenfeld et al. 2003).

Hypertrophic astrocytes are seen to surround parasite associated lesions, and astrocytes deficient in the expression of their major intermediate filament GFAP have increased parasite load, widespread inflammation, and a reduced capacity to restrict the lesion (Stenzel et al. 2004). This highlights the immunoregulatory function of astrocytes in toxoplasma encephalitis. Astrocyte gp130 expression is critical for the control of toxoplasma encephalitis, which is a signal transducer for

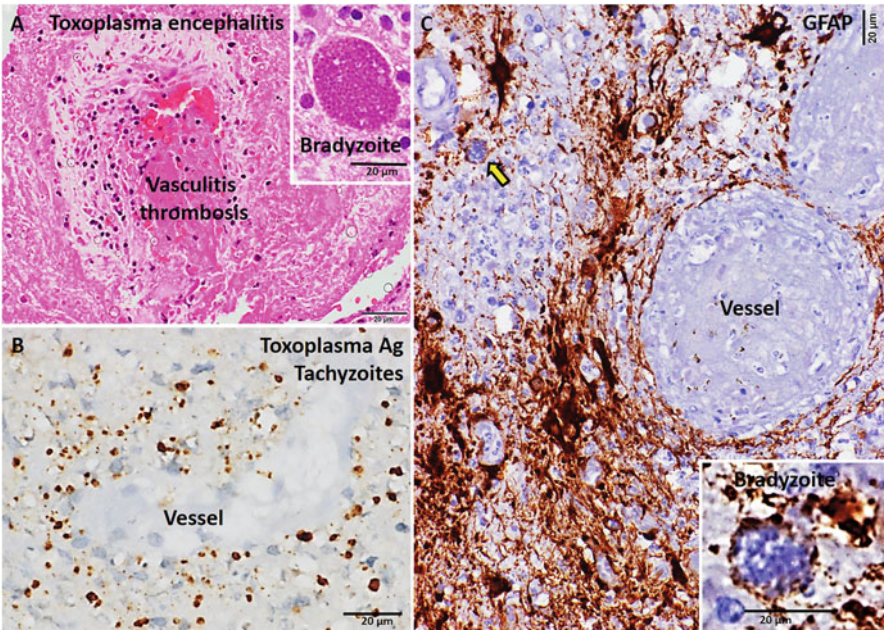

Fig. 11 Toxoplasma encephalitis shows the characteristic bradyzoite forms of T. gondii within the CNS which represent the latent stage (inset, **a**). During active infection the bradyzoites rupture to release tachyzoites which have an affinity to the endothelial cells leading to vasculitis with obliterative endarteritis and thrombosis, eventually leading to hemorrhagic necrosis (**a**). The necrosis is typically dirty with presence of hematoxyphilic bodies, some of which represent extracellular tachyzoites (2–4 µm in size). IHC to toxoplasma antigen highlights the tachyzoites (**b**). GFAP shows perilesional reactive astrogliosis (**c**). The glial processes are also seen surrounding the individual bradyzoites (inset, **c**)

members of the IL-6 cytokine family (Drogemuller et al. 2008). Mice deficient in IL-6 show enhanced susceptibility to toxoplasmosis (Suzuki et al. 1997).

Cerebral Malaria (CM)

CM, caused by Plasmodium falciparum, is not an encephalitis. Its pathogenesis lies in the sequestration of parasitized red blood cells (PRBC) within the cerebral microvasculature. The PRBCs are easily recognized by the intraerythrocytic pigment hemozoin, which is the breakdown product of hemoglobin, which is catabolized and utilized by the parasite for nutrition (Lucas 2015). Experimental studies have shown that the hemozoin pigment is taken up by the human neurons and astrocytes, resulting in cellular dysfunction, toxicity, dysregulation of proapoptotic proteins, and subsequent apoptosis (Eugenin et al. 2019). Astrocyte death and breakdown of BBB due to the action of various matrix metalloproteinase released from activated glial cells leads to cerebral edema (Szklarczyk et al. 2007). Hypoxia induced by sequestration of PRBCs and cytokine expression in the brain are two primary mechanisms thought to contribute to the pathogenesis of CM. Human and murine

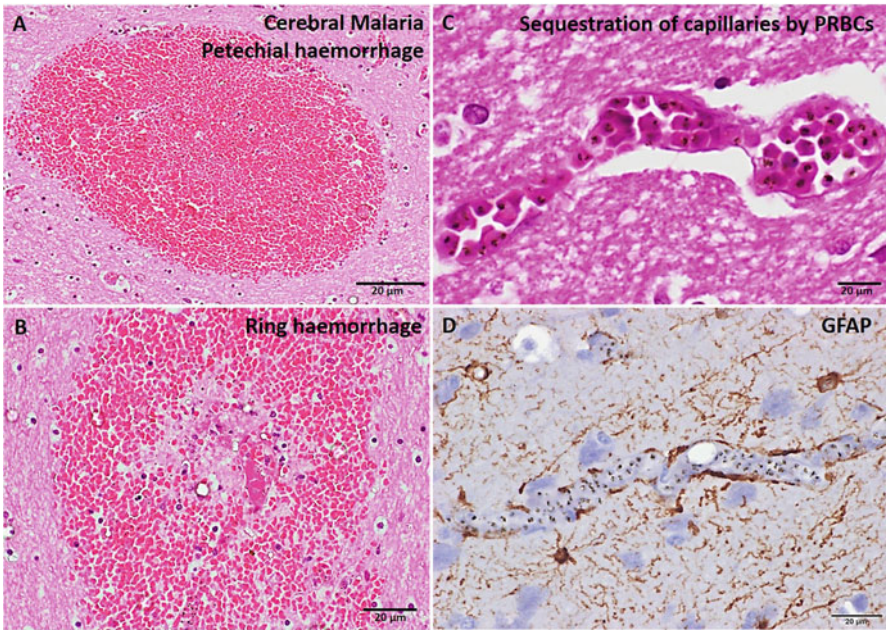

Fig. 12 Cerebral malaria is characterized by sequestration of the capillaries by parasitized red blood cells (PRBCs) which show intraerythrocytic brown hemozoin pigment (**c**). Sequestration leads to local hypoxia resulting to petechial hemorrhages which are predominantly seen in the white matter (**a**). More specific to cerebral malaria is the ring hemorrhage, consisting of a central necrosed blood vessel with fibrin thrombus surrounded by concentric zones of PRBCs and uninfected erythrocytes (**b**). GFAP shows perivascular reactive stellate astrocytes with prominent astrocytic foot processes enveloping the capillaries with PRBCs (**d**)

astrocytes in vitro have shown to contribute to CM pathogenesis by producing CXCL10 in response to IFN-γ and LT-α. CXCL10 plays an essential role in CM by attracting immune cells, such as CD8+ T lymphocytes which damage the integrity of the BBB (Bakmiwewa et al. 2016).

Durck's granulomas seen on histology in CM are characterized by aggregates of reactive astrocytes, microglial cells, and some lymphocytes. Reactive astrocytes proximal to Durck's granulomas show cell surface expression of urokinase plasminogen activator receptor whereas quiescent astrocytes do not. This contributes to BBB disintegration, immune cell recruitment, parasite adhesion and reorganization process such as clearance of infected and uninfected erythrocytes from hemorrhages (Fauser et al. 2000).

Neurocysticercosis (NCC)

NCC is parasitic infection of the CNS, caused by ingestion of eggs of the tapeworm Taenia solium with seizure as the most common presenting symptom. However not all cases can be effectively managed by anti-seizure drugs and some evolve into drug

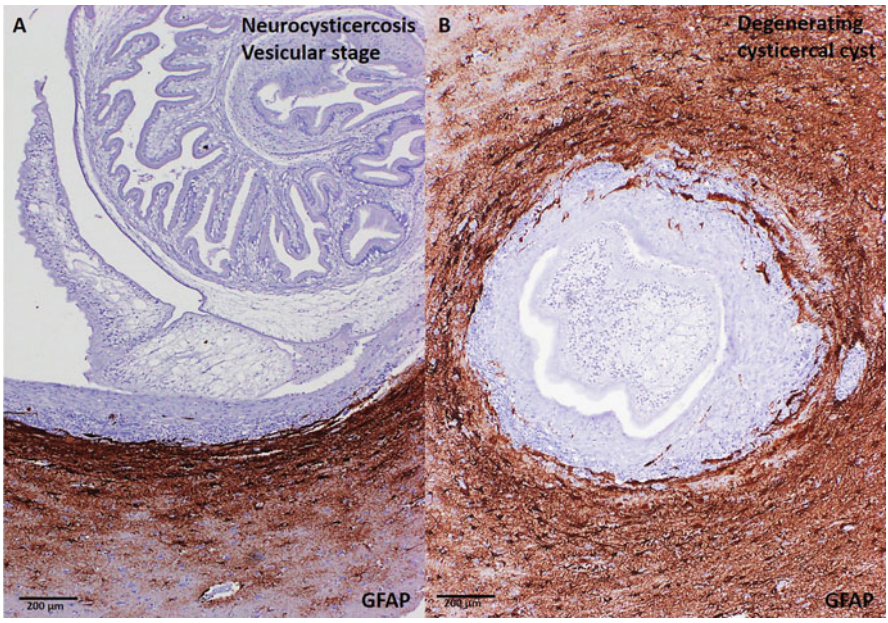

Fig. 13 Neurocysticercosis caused by larval forms of T. solium are seen in various stages within the CNS, from the active vesicular stage to the degenerating colloid granular nodular stage and the dead calcified form. The gliosis in the active stage is mild (**a**). With degeneration as the parasite antigen is exposed, more gliosis sets in forming a dense band surrounding the cyst with multilayering of astrocyte processes, some of them entering the degenerated cyst (**b**). Gradually the cyst calcifies; however, in some cases the perilesional gliosis persists which is implicated to be causal for drug resistant epilepsy

resistant epilepsy. Neuroimaging studies report hyperintense signal at the site of the involuting cysticercus reflecting perilesional gliosis, which is associated with increased seizure recurrence (Singh and Sander 2018; Rathore et al. 2013). T1-weighted magnetization transfer spin-echo sequence is useful for recognition of perilesional gliosis associated with NCC, which appears to be the reason for refractory epilepsy. Studies have confirmed this perilesional gliosis on histopathology as the cause for epileptogenicity (Escalaya and Burneo 2017, Suller-Marti et al. 2019). The antigens entrapped within the dead calcified cyst may be released by partial decalcification or micromodeling of the lesion, which triggers host immune response. This chronic inflammation triggers hyperplastic change in astrocytes leading to gliosis. Changes in the astroglial structure and properties leads to reduced extracellular buffering of electrolytes such as K+ with altered chemical extracellular environment, which eventually influences the neuronal activity. Increased intercellular glial coupling by gap junctions in the perilesional gliotic area may cause synchronization of discharges and spread of seizure activity leading to ineffective antiepileptic drug therapy (Mhatre et al. 2020; Pradhan et al. 2000).

7 Conclusion

Astrocytes the resident immunocompetent cells of CNS and one of first line of defense to infective pathogens respond in a unique manner depending not only upon the type of infective agent but also the host immune status. Reactive astrocytes are of various subtypes which can be either beneficial by limiting the spread of infection or harmful by activating uncontrolled self-perpetuating immune response along with the microglial cells. Discovery of astrocytic subtypes have opened up a whole new realm of enticing research which remains to be explored in the field of CNS infections with a prospect of modulating them at various stages of infection to bring about an optimal benefit while controlling the spread of the pathogen.

References

Altan-Bonnet N (2016) Extracellular vesicles are the Trojan horses of viral infection. Curr Opin Microbiol 32:77–81

Bakmiwewa SM, Weiser S, Grey M et al (2016) Synergistic induction of CXCL10 by interferon-gamma and lymphotoxin-alpha in astrocytes: possible role in cerebral malaria. Cytokine 78:79–86

Brack-Werner R (1999) Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. AIDS 13(1):1–22

Brilha S, Ong CWM, Weksler B, Romero N, Couraud PO, Friedland JS (2017) Matrix metalloproteinase-9 activity and a downregulated hedgehog pathway impair blood-brain barrier function in an in vitro model of CNS tuberculosis. Sci Rep 7(1):16031

Brissette CA, Kees ED, Burke MM, Gaultney RA, Floden AM, Watt JA (2013) The multifaceted responses of primary human astrocytes and brain microvascular endothelial cells to the Lyme disease spirochete, Borrelia burgdorferi. ASN Neuro 5(3):221–229

Cheeran MC, Hu S, Sheng WS, Rashid A, Peterson PK, Lokensgard JR (2005) Differential responses of human brain cells to West Nile virus infection. J Neurovirol 11(6):512–524

Chen CJ, Ou YC, Chang CY et al (2011) TNF-α and IL-1β mediate Japanese encephalitis virus-induced RANTES gene expression in astrocytes. Neurochem Int 58(2):234–242

Churchill MJ, Wesselingh SL, Cowley D et al (2009) Extensive astrocyte infection is prominent in human immunodeficiency virus-associated dementia. Ann Neurol 66(2):253–258

Clarke LE, Liddelow SA, Chakraborty C, Münch AE, Heiman M, Barres BA (2018) Normal aging induces A1-like astrocyte reactivity. Proc Natl Acad Sci U S A 115(8):E1896–E1905

Diniz JA, Da Rosa AP, Guzman H et al (2006) West Nile virus infection of primary mouse neuronal and neuroglial cells: the role of astrocytes in chronic infection. Am J Trop Med Hyg 75(4): 691–696

Donati D, Martinelli E, Cassiani-Ingoni R et al (2005) Variant-specific tropism of human herpesvirus 6 in human astrocytes. J Virol 79(15):9439–9448

Dotevall L, Rosengren LE, Hagberg L (1996) Increased cerebrospinal fluid levels of glial fibrillary acidic protein (GFAP) in Lyme neuroborreliosis. Infection 24(2):125–129

Drogemuller K, Helmuth U, Brunn A et al (2008) Astrocyte gp130 expression is critical for the control of toxoplasma encephalitis. J Immunol 181(4):2683–2693

Escalaya AL, Burneo JG (2017) Epilepsy surgery and neurocysticercosis: assessing the role of the cysticercotic lesion in medically-refractory epilepsy. Epilepsy Behav 76:178–181

Esen N, Shuffield D, Syed MM, Kielian T (2007) Modulation of connexin expression and gap junction communication in astrocytes by the gram-positive bacterium S. aureus. Glia 55(1): 104–117

Esen N, Tanga FY, DeLeo JA, Kielian T (2004) Toll-like receptor 2 (TLR2) mediates astrocyte activation in response to the gram-positive bacterium Staphylococcus aureus. J Neurochem 88(3):746–758

Eugenin EA, Martiney JA, Berman JW (2019) The malaria toxin hemozoin induces apoptosis in human neurons and astrocytes: potential role in the pathogenesis of cerebral malaria. Brain Res 1720:146317

Fauser S, Deininger MH, Kremsner PG et al (2000) Lesion associated expression of urokinase-type plasminogen activator receptor (uPAR, CD87) in human cerebral malaria. J Neuroimmunol 111(1–2):234–240

Fischer HG, Nitzgen B, Reichmann G, Hadding U (1997) Cytokine responses induced by toxoplasma gondii in astrocytes and microglial cells. Eur J Immunol 27(6):1539–1548

Fotheringham J, Williams EL, Akhyani N, Jacobson S (2008) Human herpesvirus 6 (HHV-6) induces dysregulation of glutamate uptake and transporter expression in astrocytes. J Neuroimmune Pharmacol 3(2):105–116

Geyer S, Jacobs M, Hsu NJ (2019) Immunity against bacterial infection of the central nervous system: an astrocyte perspective. Front Mol Neurosci 12:57

Gorry PR, Ong C, Thorpe J et al (2003) Astrocyte infection by HIV-1: mechanisms of restricted virus replication, and role in the pathogenesis of HIV-1-associated dementia. Curr HIV Res 1(4):463–473

Gras G, Porcheray F, Samah B, Leone C (2006) The glutamate-glutamine cycle as an inducible, protective face of macrophage activation. J Leukoc Biol 80(5):1067–1075

Halonen SK, Taylor GA, Weiss LM (2001) Gamma interferon-induced inhibition of toxoplasma gondii in astrocytes is mediated by IGTP. Infect Immun 69(9):5573–5576

Harris JE, Nuttall RK, Elkington PT et al (2007) Monocyte-astrocyte networks regulate matrix metalloproteinase gene expression and secretion in central nervous system tuberculosis in vitro and in vivo. J Immunol 178(2):1199–1207

Hensel N, Raker V, Förthmann B et al (2019) HSV-1 triggers paracrine fibroblast growth factor response from cortical brain cells via immediate-early protein ICP0. J Neuroinflammation 16(1): 248

Huang Y, Li Y, Zhang H et al (2018) Zika virus propagation and release in human fetal astrocytes can be suppressed by neutral sphingomyelinase-2 inhibitor GW4869. Cell Discov 4:19

Hussmann KL, Samuel MA, Kim KS, Diamond MS, Fredericksen BL (2013) Differential replication of pathogenic and nonpathogenic strains of West Nile virus within astrocytes. J Virol 87(5): 2814–2822

Hussmann KL, Vandergaast R, Zheng K, Hoover LI, Fredericksen BL (2014) Structural proteins of West Nile virus are a major determinant of infectious particle production and fitness in astrocytes. J Gen Virol 95:1991–2003

John Lin CC, Yu K, Hatcher A et al (2017) Identification of diverse astrocyte populations and their malignant analogs. Nat Neurosci 20(3):396–405

Kramer-Hämmerle S, Rothenaigner I, Wolff H, Bell JE, Brack-Werner R (2005) Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. Virus Res 111(2):194–213

Lee SC, Dickson DW, Brosnan CF, Casadevall A (1994) Human astrocytes inhibit Cryptococcus neoformans growth by a nitric oxide-mediated mechanism. J Exp Med 180(1):365–369

Lee SC, Dickson DW, Casadevall A (1996) Pathology of cryptococcal meningoencephalitis: analysis of 27 patients with pathogenetic implications. Hum Pathol 27(8):839–847

Lewis RE, Wiederhold NP, Lionakis MS, Prince RA, Kontoyiannis DP (2005) Frequency and species distribution of gliotoxin-producing aspergillus isolates recovered from patients at a tertiary-care cancer center. J Clin Microbiol 43(12):6120–6122

Li F, Wang Y, Yu L et al (2015) Viral infection of the central nervous system and Neuroinflammation precede blood-brain barrier disruption during Japanese encephalitis virus infection. J Virol 89(10):5602–5614

Li T, Chen X, Zhang C et al (2019) An update on reactive astrocytes in chronic pain. J Neuroinflammation 16:140
Li T, Liu T, Chen X et al (2020) Microglia induce the transformation of A1/A2 reactive astrocytes via the CXCR7/PI3K/Akt pathway in chronic post-surgical pain. J Neuroinflammation 17(1): 211
Li W, Henderson LJ, Major EO, Al-Harthi L (2011) IFN-gamma mediates enhancement of HIV replication in astrocytes by inducing an antagonist of the beta-catenin pathway (DKK1) in a STAT 3-dependent manner. J Immunol 186(12):6771–6778
Liddelow SA, Barres BA (2017) Reactive astrocytes: production, function, and therapeutic potential. Immunity 46(6):957–967
Liddelow SA, Guttenplan KA, Clarke LE et al (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541(7638):481–487
Lindqvist R, Mundt F, Gilthorpe JD et al (2016) Fast type I interferon response protects astrocytes from flavivirus infection and virus-induced cytopathic effects. J Neuroinflammation 13(1):277
Liu T, Zhang L, Joo D, Sun SC (2017) NF-κB signaling in inflammation. Signal Transduct Target Ther 2:17023
Lokensgard JR, Cheeran MC, Gekker G, Hu S, Chao CC, Peterson PK (1999) Human cytomegalovirus replication and modulation of apoptosis in astrocytes. J Hum Virol 2(2):91–101
Lokensgard JR, Cheeran MC, Hu S, Gekker G, Peterson PK (2002) Glial cell responses to herpesvirus infections: role in defense and immunopathogenesis. J Infect Dis 186(Suppl 2): S171–S179
Lokensgard JR, Hu S, Sheng W et al (2001) Robust expression of TNF-alpha, IL-1beta, RANTES, and IP-10 by human microglial cells during nonproductive infection with herpes simplex virus. J Neurovirol 7(3):208–219
Love S, Wiley CA, Lucas S (2015) Viral infections. In: Love S, Perry A, Ironside J, Budka H (eds) Greenfield's neuropathology, 9th edn. CRC Press, New York, pp 1087–1191
Lucas S (2015) Parasitic infections. In: Love S, Perry A, Ironside J, Budka H (eds) Greenfield's neuropathology, 9th edn. CRC Press, New York, pp 1230–1280
Mazlo M, Tariska I (1982) Are astrocytes infected in progressive multifocal leukoencephalopathy (PML)? Acta Neuropathol 56(1):45–51
Meertens L, Labeau A, Dejarnac O et al (2017) Axl mediates ZIKA virus entry in human glial cells and modulates innate immune responses. Cell Rep 18(2):324–333
Mhatre R, Poyuran R, Arimappamagan A et al (2020) Dual/double pathology in neurocysticercosis causing drug resistant epilepsy—chance association or causal? Epilepsy Res 168:106472
Miller SJ (2018) Astrocyte heterogeneity in the adult central nervous system. Front Cell Neurosci 12:401
Mlakar J, Korva M, Tul N et al (2016) Zika virus associated with microcephaly. N Engl J Med 374(10):951–958
Monel B, Compton AA, Bruel T et al (2017) Zika virus induces massive cytoplasmic vacuolization and paraptosis-like death in infected cells. EMBO J 36(12):1653–1668
Palus M, Bíly T, Elsterova J et al (2014) Infection and injury of human astrocytes by tick-borne encephalitis virus. J Gen Virol 95:2411–2426
Patel R, Hossain MA, German N, Al-Ahmad AJ (2018) Gliotoxin penetrates and impairs the integrity of the human blood-brain barrier in vitro. Mycotoxin Res 34(4):257–268
Perides G, Tanner-Brown LM, Eskildsen MA, Klempner MS (1999) Borrelia burgdorferi induces matrix metalloproteinases by neural cultures. J Neurosci Res 58(6):779–790
Persidsky Y, Poluektova L (2006) Immune privilege and HIV-1 persistence in the CNS. Immunol Rev 213:180–194
Potokar M, Jorgacevski J, Zorec R (2019) Astrocytes in Flavivirus infections. Int J Mol Sci 20(3): 691
Potokar M, Korva M, Jorgacevski J, Avsic-Zupanc T, Zorec R (2014) Tick-borne encephalitis virus infects rat astrocytes but does not affect their viability. PLoS One 9(1):e86219

Potokar M, Kreft M, Li L et al (2007) Cytoskeleton and vesicle mobility in astrocytes. Traffic 8(1): 12–20

Pradhan S, Kathuria MK, Gupta RK (2000) Perilesional gliosis and seizure outcome: a study based on magnetization transfer magnetic resonance imaging in patients with neurocysticercosis. Ann Neurol 48(2):181–187

Raivich G, Bohatschek M, Kloss CU, Werner A, Jones LL, Kreutzberg GW (1999) Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. Brain Res Brain Res Rev 30(1):77–105

Ramesh G, Alvarez AL, Roberts ED et al (2003) Pathogenesis of Lyme neuroborreliosis: Borrelia burgdorferi lipoproteins induce both proliferation and apoptosis in rhesus monkey astrocytes. Eur J Immunol 33(9):2539–2550

Rathore C, Thomas B, Kesavadas C, Abraham M, Radhakrishnan K (2013) Calcified neurocysticercosis lesions and antiepileptic drug-resistant epilepsy: a surgically remediable syndrome? Epilepsia 54(10):1815–1822

Retallack H, Di Lullo E, Arias C et al (2016) Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proc Natl Acad Sci U S A 113(50):14408–14413

Rock RB, Hu S, Gekker G et al (2005) Mycobacterium tuberculosis-induced cytokine and chemokine expression by human microglia and astrocytes: effects of dexamethasone. J Infect Dis 192(12):2054–2058

Rozenfeld C, Martinez R, Figueiredo RT et al (2003) Soluble factors released by toxoplasma gondii-infected astrocytes down-modulate nitric oxide production by gamma interferon-activated microglia and prevent neuronal degeneration. Infect Immun 71(4):2047–2057

Sejvar JJ (2018) Zika virus and other emerging Arboviral central nervous system infections. Continuum (Minneap Minn) 24:1512–1534

Shao Q, Herrlinger S, Yang SL et al (2016) Zika virus infection disrupts neurovascular development and results in postnatal microcephaly with brain damage. Development 143(22):4127–4136

Singh G, Sander JW (2018) Neurocysticercosis as a probable risk factor for hippocampal sclerosis. Arq Neuropsiquiatr 76(11):783–790

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32(12):638–647

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119(1): 7–35

Spanos JP, Hsu NJ, Jacobs M (2015) Microglia are crucial regulators of neuro-immunity during central nervous system tuberculosis. Front Cell Neurosci 9:182

Speth C, Rambach G, Lass-Florl C, Wurzner R, Gasque P, Mohsenipour I, Dierich MP (2000) Culture supernatants of patient-derived aspergillus isolates have toxic and lytic activity towards neurons and glial cells. FEMS Immunol Med Microbiol 29(4):303–313

Stefanik M, Formanova P, Bily T et al (2018) Characterisation of Zika virus infection in primary human astrocytes. BMC Neurosci 19(1):5

Stenzel W, Soltek S, Schlüter D, Deckert M (2004) The intermediate filament GFAP is important for the control of experimental murine Staphylococcus aureus-induced brain abscess and toxoplasma encephalitis. J Neuropathol Exp Neurol 63(6):631–640

Strack A, Asensio VC, Campbell IL, Schlüter D, Deckert M (2002) Chemokines are differentially expressed by astrocytes, microglia and inflammatory leukocytes in toxoplasma encephalitis and critically regulated by interferon-gamma. Acta Neuropathol 103(5):458–468

Suller-Marti A, Escalaya AL, Burneo JG (2019) Epilepsy surgery for refractory neurocysticercosis-related epilepsy. Rev Neurol 68(9):384–388

Suzuki Y, Conley FK, Remington JS (1989) Importance of endogenous IFN-gamma for prevention of toxoplasmic encephalitis in mice. J Immunol 143(6):2045–2050

Suzuki Y, Rani S, Liesenfeld O et al (1997) Impaired resistance to the development of toxoplasmic encephalitis in interleukin-6-deficient mice. Infect Immun 65(6):2339–2345

Szklarczyk A, Stins M, Milward EA et al (2007) Glial activation and matrix metalloproteinase release in cerebral malaria. J Neurovirol 13(1):2–10

Taylor GA, Collazo CM, Yap GS et al (2000) Pathogen-specific loss of host resistance in mice lacking the IFN-gamma-inducible gene IGTP. Proc Natl Acad Sci U S A 97(2):751–755

Tripathi S, Patro I, Mahadevan A, Patro N, Phillip M, Shankar SK (2014) Glial alterations in tuberculous and cryptococcal meningitis and their relation to HIV co-infection--a study on human brains. J Infect Dev Ctries 8(11):1421–1443

van Marle G, Antony J, Ostermann H et al (2007) West Nile virus-induced neuroinflammation: glial infection and capsid protein-mediated neurovirulence. J Virol 81(20):10933–10949

Vinters HV, Kleinschmidt-DeMasters HV (2015) General pathology of central nervous system. In: Love S, Perry A, Ironside J, Budka H (eds) Greenfield's neuropathology, 9th edn. CRC Press, New York, pp 1–58

Vismara I, Papa S, Veneruso V et al (2020) Selective modulation of A1 astrocytes by drug-loaded Nano-structured gel in spinal cord injury. ACS Nano 14(1):360–371

Voskuhl RR, Peterson RS, Song B et al (2009) Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. J Neurosci 29(37): 11511–11522

Wainwright MS, Martin PL, Morse RP et al (2001) Human herpesvirus 6 limbic encephalitis after stem cell transplantation. Ann Neurol 50(5):612–619

Wilczek MP, DuShane JK, Armstrong FJ, Maginnis MS (2020) JC polyomavirus infection reveals delayed progression of the infectious cycle in Normal human astrocytes. J Virol 94(5):e01331–e01319

Wuthrich C, Batson S, Anderson MP, White LR, Koralnik IJ (2016) JC virus infects neurons and glial cells in the hippocampus. J Neuropathol Exp Neurol 75(8):712–717

Xu X, Zhang A, Zhu Y et al (2018) MFG-E8 reverses microglial-induced neurotoxic astrocyte (A1) via NF-κB and PI3K-Akt pathways. J Cell Physiol 234(1):904–914

Zamanian JL, Xu L, Foo LC et al (2021) Genomic analysis of reactive astrogliosis. J Neurosci 32(18):6391–6410

Role of Reactive Astrocytes in Alzheimer's Disease

Sukanya Sarkar, Subhalakshmi Guha, and Subhas C. Biswas

Abstract

Astrocytes respond to any pathological stimulus to the central nervous system including in Alzheimer's disease (AD). They undergo dramatic remodeling at the molecular, cellular, and functional levels to constitute a heterogeneous population across disease stages that are collectively termed as reactive astrocytes. "Astrocyte reactivity" or "reactive astrogliosis" encompasses multiple distinct states astrocytes adopt across the disease stages. For several decades this phenomenon has been considered a nonspecific reaction to pathological insults without any disease-inducing mechanisms and with no therapeutic value. Recent studies have contrarily underscored the specific roles of astrocytes in disease pathogenesis. With the advent of single-cell and single-nucleus transcriptomics, numerous disease-modifying functions of reactive astrocytes are revealed. Diverse subtypes of reactive astrocytes are currently the major focus of AD research. Previously astrocytes were thought to contribute towards neuronal degeneration by releasing pro-inflammatory mediators in AD. Present evidences indicate that reactive astrocytes also play a pivotal role in neuroprotection, plausibly at the prodromal AD stages by secreting anti-inflammatory molecules, clearing Aβ and limiting neuroinflammation in the CNS. In this chapter we attempt to review the extensive yet subtle functional diversities in reactive astrocytes in AD with respect to metabolic alterations, neuroinflammation, Aβ production and clearance, tau pathology, synaptic plasticity, neurotransmitter recycling, and their impact on neuronal health. We further highlight their contribution in identifying early-stage AD biomarkers. Thus, reactive astrocytes may represent an attractive therapeutic target in halting AD progression, its prevention, or in cure.

S. C. Biswas (✉)
Cell Biology and Physiology Division, CSIR-Indian Institute of Chemical Biology, Kolkata, West Bengal, India; subhasbiswas@iicb.res.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_9

Keywords

Alzheimer's disease · Reactive astrogliosis · Astrocyte · Neuroinflammation · Aβ · Synaptic plasticity · Astrocyte reactivity · Early-stage AD biomarkers · Neurotransmitter recycling · Tau pathology

1 Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder of aged individuals that presents a huge socioeconomic burden and challenges the global health system. AD patients lose memory, learning and thinking skills, and other cognitive functions leading to irrational behaviors. The drugs available for AD to date only provide symptomatic relief at the early stages. There are no confirmatory early diagnostic tests or disease modifying therapies. The major obstacle in the development of therapies for AD is poor understanding of the fundamental basis of the disease pathogenesis. The past several decade-long AD researches held a neuron-centric view of AD pathogenesis and have failed to translate into effective therapeutic strategies. This failure reflects the lack of understanding of pathological alterations in glia beyond the old-concept of glial cells as the "brain glue." Astrocytes are not only an integral part of the neural system but are also equally significant in the cell-cell interactions and related perturbations occurring during disease pathogenesis (Long and Holtzman 2019).

Available literature elaborates the role of inflammation in neurodegeneration, either as a factor that sustains the degenerative process or as playing a significant disease initiating role. The brain contains an endogenous "innate immune system," primarily microglia/astrocytes, and also has an ability to recruit the peripheral immune system especially in the wake of any trauma. Under physiological conditions, astrocytes supply energy to neurons, maintain synaptic functions and ionic balance, recycle excess glutamate to prevent glutamate excitotoxicity, and are a major source of antioxidants such as ascorbic acid and glutathione (Belanger and Magistretti 2009). However, in most central nervous system (CNS) insult or disease, astrocytes transform morphologically, transcriptionally, biochemically, and functionally through a process called astrocyte reactivity or reactive astrogliosis—a phenomenon characterized by hypertrophy and the secretion of a pool of pro- and anti-inflammatory mediators such as cytokines and chemokines (Olabarria et al. 2010; Sofroniew 2014a). Current literature suggests that reactive astrocytes can exist in several distinct molecular states in many diseases and across different regions of the brain (Das et al. 2020; Matias et al. 2019). Coherently, in AD and other neurodegenerative diseases, astrocytes attain several distinct reactive phenotypes with varied impacts on disease progression (Habib et al. 2020).

Earlier it was thought that the reactive astrogliosis in neurodegenerative diseases was largely associated with irreversible alterations in astrocytes that contributed to an overall CNS deterioration and exacerbated disease state. Currently, astrocyte reactivity is being seen in the light of neuroprotective abilities that promote neuronal

health. In this chapter, we discuss both the offensive and defensive aspects of this phenomenon. We highlight the major pathways and the critical proteins/cytokines involved in this dynamic process. We try to delineate the stage-specific reactive astrocyte secretomes and their role in mediating astrocytes' functions from early to the later stages in AD.

2 Astrocytes in AD

This chapter will mostly focus on understanding the role of astrocytes, as a key player among the nonneuronal cells in the pathogenesis of AD through a detailed report on its types and subtypes, its role in the initiation of neuroinflammation in AD, in Aβ generation and clearance; tau pathology; metabolic dysregulation; synaptic plasticity; neurotransmitter recycling; and neuron death and survival. The neuron-centric perspective of the pathogenesis of AD has largely failed in terms of translational research since no effective drug has been developed for AD till date. So in a fresh approach, neurons must be studied as an integrated part of a community of nonneuronal cells which are all victims of the initial pathological insult in AD and how among them astrocytes play a dominant role in recovering the health of the brain and their subsequent failure leading to disease progression. It is now recognized that astrocytes have an essential role in the brain as well as are heavily involved in the pathogenesis and progression in AD. Thus understanding them will further pave the way for the development of important early stage AD biomarkers or novel targets for therapy.

2.1 Types and Subtypes

The astrocyte population is heterogeneous. It would be thus interesting to learn how a shift from the physiological balance to the disease state affect them in terms of phenotype, behavior, cellular signaling, and intercellular interactions. Astrocytes elicit distinct effects in response to plaques and neurofibrillary tangles, aggregated Aβ, and hyperphosphorylated tau in AD, which maybe neuroprotective or detrimental.

Physiological Subtypes

Astrocytes account for 20–40% of all neuroglial cells based on the brain region. Astrocyte, named so for its stellate morphology, has been long debated for its constitution given the diversity in its types as well as the similarities it shares with other cell types in the CNS. The diversity of astrocytes is summarized in a review by Verkhratsky et al. (2019b) and has been discussed in previous chapters.

Astrogliopathology

A basic involvement of astrocytes in neuronal pathology was suggested by Santiago Ramon y Cajal, Alois Alzheimer, Franz Nissl, Pio del Rio Hortega, and William

Lloyd Andriezen, the latter indicating that the astrocytes "exhibit a morbid hypertrophy in pathological conditions" (Andriezen 1893). Pathological alternations in the morphology of astrocytes are very complex and are disease-stage specific. They are subject to changes in the course of disease progression and evolution as depicted by Verkhratsky et al. (2019b). As mentioned by the group, the three major categories portrayed on the basis of phenotypes of astrogliopathology are—(a) reactive astrogliosis, (b) astrodegeneration with astroglial atrophy and loss of function, and (c) pathological remodeling (Pekny et al. 2016; Verkhratsky et al. 2017). Excluding reactive astrogliosis, the other two groups are together categorized as astrocytopathies.

Astrocyte Reactivity or Reactive Astrogliosis

Astrocytes undergo remarkable alterations morphologically, transcriptionally, biochemically, and functionally to generate a heterogeneous population in response to any CNS insult and are collectively termed as reactive astrocytes. An agglomeration of multidimensional data based on transcriptomic and proteomic studies indicate extensive yet subtle functional diversities in reactive astrocytes that impact the disease progression in disparate ways (Anderson et al. 2014). Astrocytes are considered as one of the first responders to a pathological insult to the brain that undergo huge molecular and functional metamorphoses as mentioned above, yet some of those alterations may resolve while others persist (Escartin et al. 2019). Recently, in a consensus report from glial biologists across the globe, reactive astrogliosis, more accurately astrocyte reactivity is defined as a highly complex disease-stage and disease-specific phenomenon encompassing all the potential reactive states of astrocytes where "state" may be defined as a temporary or permanent remodeling of astrocytes (Escartin et al. 2021). Astrocyte reactivity is a response to pathological insult and may be classified based on the nature of triggering stimulus which may be external in nature or cell-autonomous in its generation (Fig. 1). Upregulated expressions of glial fibrillary acidic protein (GFAP), Nestin, Vimentin, or s100β proteins indicating the presence of hypertrophic astrocytes are heavily detected in AD postmortem brains (Verkhratsky et al. 2016). They are often found in association with senile plaques representing a transcriptomic profile dominated by pro-inflammatory cytokines both in rodent AD models and in human AD patients (Li et al. 2011a). Reactive astrocytes are also seen as the driving force behind the neuroinflammatory processes in AD such that inhibition of astrocytic activation in APP/PS1mice attenuated neuroinflammation while improving cognitive functions and reducing Aβ plaque deposition (refer Table 1 for details) (Furman et al. 2012). Pro-inflammatory cytokines released by reactive astrocytes can indeed act through autocrine signaling to increase the synthesis and release of Aβ from the reactive astrocytes themselves (Blasko et al. 2000), besides reducing microglial Aβ phagocytosis (Koenigsknecht-Talboo and Landreth 2005), or they may act on neurons directly increasing caspase-3 activity, altering tau phosphorylation and eventually leading to neuronal death (Garwood et al. 2011).

In other cases, reactive astrocytes were detected prior to the appearance of amyloid plaques (Wang et al. 2018) and inhibiting astrocyte reactivity propagated

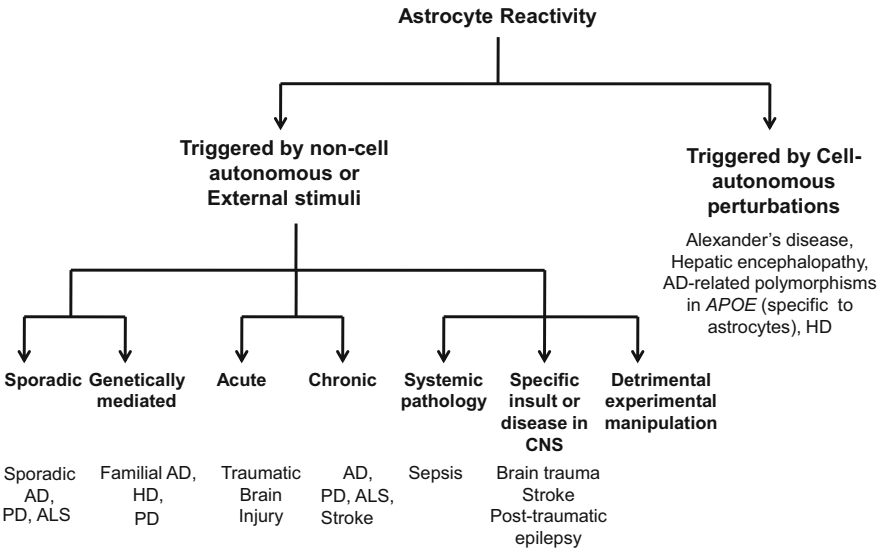

Fig. 1 Classification of Astrocyte reactivity based on the nature of stimuli: Astrocyte reactivity is defined as astrocytes' reaction to an insult (Escartin et al. 2021). The accepted terminology is "astrocyte reactivity" and is used to distinguish the astrocytes responding to a pathological insult from "astrocyte activation" which defines astrocyte plasticity in response to various physiological stimuli in the CNS. We have indicated the two types of triggering stimuli that induce astrocyte reactivity and given some examples of associated diseases in each subcategory. AD, Alzheimer's disease; PD, Parkinson's disease; ALS, amyotrophic lateral sclerosis; HD, Huntington's disease

amyloid pathology in a mouse model of AD (Kraft et al. 2013). These data allude to a neuroprotective ability of the reactive astrocytes in facilitating Aβ clearance especially in early disease stages (Saha et al. 2020). Thus, reactive astrogliosis is not a straight pathological reaction that leads to brain damage; instead, it can be thought as an initial defensive mechanism to protect the neurons from further damage. Notably, domain organization of reactive astrocytes is not lost in AD (Oberheim et al. 2008), and astrocytic proliferation is only rarely detected in AD animal models (Sirko et al. 2013). Thus, a mild astrogliotic response is initially induced in AD by several potential molecules, namely, Aβ or certain cytokines and chemokines or molecules secreted from injured cells. With the emergence of transcriptome profiling as a major tool to distinguish certain type of reactive astrocytes on the basis of specific genes other than *Gfap* from the rest, Zamanian et al. (2012) and Liddelow and Barres (2017) first profiled pure reactive astrocytes. They showed that upon treatment of a group of mice with lipopolysaccharide (LPS) that induces a neuroinflammation model and by occluding middle cerebral artery (MCAO) in others producing an ischemic model, gene profiles of the reactive astrocytes in each of these two models were strikingly different. This work was further explained by Liddelow and Barres (2017) who first named these two subtypes of reactive astrocytes as A1 and A2, in LPS-induced brain inflammation

Table 1 Details about the transgenic mice models of Alzheimer's disease

Transgenic models in Alzheimer's disease (mentioned in this chapter)		
Transgenic mouse model	Mutations	Characteristic pathological phenotypes
Tg2576 (APPswe)	APP (isoform 695) KM670/671NL (Swedish)	Parenchymal Aβ plaques observed by 11–13 months in the neocortex and hippocampal regions. Tends to collect elevated levels of Aβ and display age-related extensive cognitive deficits. Limited or no tangles (Hsiao et al. 1996)
APP/PS1	APP KM670/671NL (Swedish) + PSEN1 L166P	Aβ plaque deposition begins in the neocortex at around 6 weeks of age. Neuritic processes showing phosphorylated tau detected near the amyloid deposits, but there were no fibrillar tau inclusions (Jankowsky et al. 2004)
ArcAβ	APP KM670/671NL (Swedish) + APP E693G (Arctic)	At around 6 months, amyloid pathology impacting both brain parenchyma and vasculature is observed. In the parenchyma, Aβ pathology begins as punctated intracellular Aβ deposits in the hippocampus and cortex (Knobloch et al. 2007)
Tg-ArcSwe	APP KM670/671NL (Swedish) + APP E693G (Arctic)	Significant Aβ pathology, with enhanced levels of soluble Aβ aggregates including Aβ protofibrils, an increased accumulation of Aβ inside neurons, and more detectable senile plaques than commonly seen with Swedish mutation alone. Intraneuronal Aβ at 1 month while extracellular plaques at 5 months (Lord et al. 2006)
3xTg	APP KM670/671NL (Swedish) + MAPT P301L + PSEN1 M146V	Fertile, viable, and does not show any gross physical or behavioral anomalies in the beginning but progressively develops plaques and tangles in an age-associated manner. Extracellular Aβ deposits at 6 months in the frontal cortex, extensive plaques, and tau pathology at 12 (Oddo et al. 2003)

(probably detrimental) and in MCAO (probably beneficial) models, respectively. They also tracked the A1-reactive phenotype to AD (Liddelow et al. 2017). The review further indicates that there might be more than the abovementioned two states of reactive astrocytes and that it is a subject of future research. A1 reactive astrocytes activate several genes of the complement cascade that have previously been reported to be deleterious to synapses including C3 protein through activation of NFκB pathway besides several other proinflammatory genes including SSP1, CXCL1, CXCL2, Serping1, H2-D1, Ggta1, etc. (Zamanian et al. 2012). In contrast, A2 astrocytes expressed elevated levels of several neurotrophic factors some of which play significant roles in anti-oxidative pathways including arginase-1, Nrf2, PTX3, SPHK1, and TM4SF1 (Zamanian et al. 2012) promoting neuronal survivability as

well as inducing thrombospondins that promote synapse repair. They have also raised the question that A1 and A2 might represent the extreme ends of a continuum of reactive astrocytes. The authors have also expressed their doubt about this heterogeneity which may or may not be predetermined. Whether the heterogeneity is established during the developmental period that dictates individual astrocyte to activate in a specific way remains an open question. More recent studies utilizing single-cell and single-nucleus RNAseq reveal that only a subset of reactive astrocytes identifiable by a mixture of A1 and A2 or pan reactive transcripts are increased manifold in mice and human brains along neurodegenerative disease progression including in AD (Grubman et al. 2019; Zhou et al. 2020; Habib et al. 2020). It is apparent that several reactive phenotypes of astrocytes exist beyond those identified to date. It would be thus really interesting to identify the key cellular switch that converts these reactive astrocytes to each of the distinct reactive states. A recent kinetic study revealed the secretomes of Aβ-activated astrocytes in primary culture model (Saha et al. 2020). Intriguingly, the early-hour Aβ activated astrocytes secreted cytokines and chemokines that are commonly associated with neuroprotection such as tissue inhibitor of metalloproteinase 1 (TIMP-1), intercellular adhesion molecule-1 (sICAM-1), cytokine-induced neutrophil chemoattractant 1 (CINC-1/CXCL1), macrophage inflammatory protein-1 alpha (MIP-1α/CCL3), and interleukin-2 (IL-2) and indeed the astrocyte-conditioned medium was found to protect the neurons against Aβ toxicity in cortical culture AD model. The late-hour astrocyte-conditioned medium comprised of an upregulated level of pro-inflammatory cytokines, namely, fractalkine and CINC-2α/β, and they were shown to be detrimental towards neurons. Hence, it may be derived that the early time point reactive astrocytes may represent A2-like (A1-like term is subsequently used here to indicate all the neurotoxic astrocyte states and A2-like to indicate neuroprotective phenotypes; not related to A1/A2 nomenclature by Barres group) phenotype while at the late time points, they mirror the A1-like phenotype. However, this theory requires further investigation. Taking cue from their proposal and the recent work from Saha et al.'s group, it may be hypothesized that in AD, the A2-like astrocytes actually precedes the A1-like astrocytes where the initial failure in protection by A2-like can lead to AD progression. Thus presence of A2-like, with its distinct gene/secretion profile, can be used as an early stage indicator in various models of AD. Further investigation of its gene/secretion profile may divulge novel therapeutic targets.

Astroglial Atrophy

There is evidence of astrocyte atrophy besides gliosis in AD transgenic mice particularly at early stages of disease progression thus indicating the significance of loss of function and atrophy besides gain of function and hypertrophy. The disintegration of calpain-positive glial processes and the excitatory amino-acid transporter 2 (EAAT-2) loss in human tissue are evidences of this phenomenon (Garwood et al. 2017). Age-related white matter lesions also displayed loss of astrocytic processes related to dysfunctional blood-brain barrier (BBB) and serum plasma protein uptake (Tomimoto et al. 1996; Simpson et al. 2007). Several reports

indicate towards a brain region specific astroglial atrophy wherein hippocampal GFAP-positive hypertrophic astrocytes were associated with Aβ plaques, while at a distance from the plaque, at the entorhinal and prefrontal cortices, GFAP profiles were found to be atrophic (Olabarria et al. 2010). Restricting to the study of hypertrophic astrocytes and neglecting atrophic responses in astrocytes may overlook crucial alterations in AD.

2.2 Metabolic Balance and Imbalance Regulated by Astrocytes in AD

Metabolic Dysregulation in AD

Astrocytes play an inevitable role in maintaining the metabolic homeostasis of brain by supporting the neurons through neurotransmitter signaling, restoring ionic gradients and providing energy substrates and cholesterol (Cai et al. 2017). Metabolic dysregulation in the brain including altered glucose metabolism and mitochondrial function is a common feature of AD pathology leading to the development of an overall hypometabolic scenario in the CNS (Forster et al. 2012; Yao et al. 2011). Progressive loss in glucose utilization in relation to different stages of AD (De Santi et al. 2001) as well as a presymptomatic reduction in cerebral glucose metabolism in AD compared to healthy controls (Mosconi et al. 2009), especially in regions that are more vulnerable to AD pathology, present this aspect with a diagnostic significance (Rodriguez-Vieitez et al. 2016; Mosconi et al. 2008; Bero et al. 2011; Oh et al. 2016; Mosconi 2013). A recent report has emphasized on the role of astrocytes in contributing to the 18-fluorodeoxyglucose (18F-FDG) PET signaling, classically used for detecting neuronal activity and diagnosis of different stages of AD (Zimmer et al. 2017). In this hypometabolic scenario, there is a dysregulation of metabolic enzymes, mitochondrial complexes, and nutrient transporters where reactive astrocytes play a crucial role (Cai et al. 2017). Indeed Zulfiqar et al. (2019) highlighted that AD is a metabolic disease based on the "neuroenergetic hypothesis" where a hypometabolic brain condition skews the APP metabolism towards the amyloidogenic pathway from the non-amyloidogenic one. This was substantiated by reports that patients with viral infections including cytomegalovirus, Epstein-Barr virus, human herpes virus-6, as well as with diseases such as diabetes mellitus, well known to be linked to glucose dysregulation were found to be at enhanced risk for developing AD (Carbone et al. 2014; Blonz 2017; Prasad et al. 2014). This hypometabolic condition can be largely attributed to the reactivated state of astrocytes with an altered metabolism that can induce Aβ generation from astrocytes themselves (Frost and Li 2017). Numerous reports suggest that an aberrant astrocytic metabolism could trigger AD development (De Strooper and Karran 2016). Reactive astrocytes in AD present reduced metabolic support to the neurons through diminished glutamate uptake, attenuated energy supply due to compromised rate of glycolysis and subsequent lactate release, and diminished oxidative support (Zulfiqar et al. 2019). Thus Aβ accumulation, tau hyperphosphorylation, and the resulting neuronal death in AD may be the outcome of an overall failure by

astrocytes in maintaining brain homeostasis (Gong et al. 2006). An interesting theory recently proposed by Cotto et al. (2019), supported by substantial evidences, states that there is an enhanced mitochondrial metabolism in astrocytes, especially with age or under stress, which is in stark contrast to the decreasing neuronal mitochondrial metabolism under similar conditions. This indicates a shift from the anaerobic glycolysis that produces lactate towards an enhanced mitochondrial functioning within the astrocytes. This further triggers the hypometabolic state for neurons that are largely dependent on these astrocytes. Hence, neurons find themselves under metabolite deprivation finally leading the aging brain towards neurological disorders including AD. Moreover, diminished expressions of astrocytic GLUT1 and neuronal GLUT3, essential for the uptake of glucose, have been found in postmortem AD brain (Simpson et al. 1994). Similarly, it has been shown that astrocytes from transgenic ArcAβ mice (refer to Table 1 for details) have reduced expression of astrocytic GLUT1 and lactate transporters which preceded a widespread Aβ plaque deposition (Merlini et al. 2011). Exposure of cultured astrocytes to Aβ triggered their reactive states and altered their cellular metabolism. However, both increase (Allaman et al. 2010) and decrease (Parpura-Gill et al. 1997; Soucek et al. 2003; Abeti et al. 2011) in glucose utilization were noted by different groups. Conversely, impaired glycolysis in fetal astrocytes from human has been shown to stimulate Aβ aggregation (Fu et al. 2015). Metabolic dysfunction in astrocytes is thus a direct trigger for Aβ pathology in AD. An increase in activity of β- and γ- secretase within the reactive astrocytes during metabolic dysregulation leads to Aβ production. This is again associated with a reduction in Aβ clearance. Additionally, Aβ pathology maybe indirectly promoted by neuroinflammation and oxidative stress induced by the metabolic dysfunction in astrocytes (Cai et al. 2017). Reactive astrocyte-induced high levels of glucose imbalances in AD may magnify lipid peroxidation that progressively reduces the antioxidant systems, inducing elevated oxidative metabolism that hampers cell structure further promoting neuronal damage and Aβ deposition (Rojas-Gutierrez et al. 2017).

Dysregulation in Astrocytic Metabolic Enzyme Activity

The activities of major glycolytic enzymes undergo significant alterations in AD. Glucose-6-phosphate dehydrogenase (G6PDH) showed decreased activity in the hippocampal region whereas lactate dehydrogenase (LDH), phosphofructokinase (PFK), and pyruvate kinase (PK) were upregulated in the frontal and temporal cortices (Bigl et al. 1999; Cotto et al. 2019). This enhanced activity of LDH, PFK, and PK is directly correlated with GFAP levels and were found to colocalize with GFAP-positive reactive astrocytes indicating that though neuronal metabolism was decreased in AD, astrocytic metabolism was enhanced (Liang et al. 2008).

Altered Insulin Metabolism

Altered insulin metabolism forms another aspect of metabolic dysfunction in AD as reports unraveled that both insulin and insulin-like growth factor 1 (IGF-1) are reduced in AD brains (Rivera et al. 2005). Hereby, astrocytic IGF-1 signaling protects neurons from Aβ-induced toxicity and oxidative stress (Pitt et al. 2017).

Moreover, insulin is the main regulator of glycogen synthesis in astrocytes (Falkowska et al. 2015). However, in AD, glycogen synthase kinase 3 (GSK3) over-activation due to diminished insulin signaling inhibits glycogen synthase, responsible for glycogen production, leading to associative learning deficits (Duran et al. 2013).

2.3 Reactive Astrocytes in Neuroinflammation in AD

Importance of Neuroinflammation in AD

Neuroinflammation may be considered as an innate protective response in order to regulate processes triggered by an injury or disease in the CNS. However, a slight aberration in any of the components in this response may alter their fate altogether having catastrophic effects on the brain (Gonzalez-Reyes et al. 2017). AD is one such disease associated with a neuroinflammatory response that has gone awry (Ransohoff 2016). In fact, neuroinflammation, besides Aβ deposition, can dictate cognitive deterioration in AD. For instance, upon suppressing microglia and astrocyte-mediated inflammatory responses, there was a significant improvement in cognitive abilities, even in a pre-amyloid scenario (Acosta et al. 2017). It has been reported that AD mouse brains have highly upregulated levels of pro-inflammatory cytokines—TNFα, IL-6, IL-1β, and IL-1α—that were linked to an increased level of Aβ (Patel et al. 2005). AD brain displays upregulated expression of genes related to inflammation compared to age-matched controls, thus contributing to progressive neurodegeneration and cognitive loss (De Strooper and Karran 2016). Importantly, elevated levels of pro-inflammatory cytokines were produced from reactive astrocytes especially those surrounding amyloid plaques in AD patients and also in AD models (Li et al. 2011a).

Astrocytes Taking a Center Stage

Both microglia and astrocytes are involved in the multilayered complex process of neuroinflammation by secreting a diverse pool of cytokines, chemokines, and other cellular mediators (Sofroniew 2014b). These molecular mediators can regulate every component of neuroinflammation including pro-inflammatory and anti-inflammatory processes as well as responses of neuronal and nonneuronal cells to insults such as Aβ. Though microglia have been extensively studied as the major contributor in AD, the role of astrocytes in neuroinflammation is slowly but surely taking the center stage. GFAP-positive astrocytes from the cortex of APP/PS1 transgenic AD mice were subjected to transcriptional analysis which revealed a pro-inflammatory profile significantly elevated in comparison to the control mice group. Moreover, the number of genes that were induced as well as the fold-change at the expression level was more prominent in the astrocytes than the microglial cells. This affirms the astrocytes as the major contributor to the cytokine production and is the pivotal factor in mediating disease progression. This trend was fortified from transcriptomic analysis of human AD astrocytes (Orre et al. 2014a; Garwood et al. 2017). Notably, the reactivated astrocytes are the key contributors towards the

neuroinflammatory processes in AD. Furman et al. (2012) showed that adeno-associated virus (AAV)-mediated inhibition of astrocytes reactivity in APP/PS1 mice by interfering with the inflammatory calcineurin/NFAT pathway ameliorated synaptic and cognitive functions, diminished astrogliosis and reduced Aβ deposition (Furman et al. 2012). Indeed evidence suggests that Aβ can induce secretion of astrocytic proinflammatory cytokines that can eventually lead to synaptic loss and neuronal death in AD (Furman et al. 2012; Garwood et al. 2011; Iram et al. 2016). The secreted pro-inflammatory cytokines can in turn dictate the reactive astrocyte themselves to enhance their Aβ production as well as release (Blasko et al. 2000) and direct the surrounding microglial cells to reduce phagocytosis of Aβ (Koenigsknecht-Talboo and Landreth 2005). Overall, these factors ensure the propagation of a toxic cycle leading to greater Aβ plaque deposition in the CNS. Besides the calcineurin/NFAT pathway in reactive astrocytes that help propagate the inflammatory morphology in AD leading to dendritic spine loss (Norris et al. 2005; Wu et al. 2010), NF-κB pathway is triggered within reactive astrocytes by Aβ in vitro which induces enhanced expression of pro-inflammatory cytokines including IL-1 and IL-6 (Bales et al. 1998) and inducible nitric oxide synthetase (Akama et al. 1998). Reactive astrocytes secreted s100β acting in an autocrine fashion can prolong the activated state of NF-κB signaling pathway (Lam et al. 2001). Notably, as mentioned earlier C3 is a complement protein, now considered a major marker of astrocytic pro-inflammatory A1 phenotype is also a target gene of NF-κB (Liddelow et al. 2017; Perez-Nievas and Serrano-Pozo 2018). Liddelow (2017) demonstrated that reactive astrocytes with the A1 phenotype are in fact induced by three critical cytokines—IL-1α, TNFα, and C1q—released by activated microglial cells.

Blood-Brain Barrier in AD

It is widely known that BBB loses its integrity in AD (Chakraborty et al. 2017) and thus contributes to neuroinflammation. Aβ accumulation in the blood vessels of AD brain and the associated vascular inflammation induces a cross communication between the brain and the peripheral nervous system (Takeda et al. 2014). Astrocyte end-feet being a major constitutive component of the BBB may be considered as a critical member in the BBB-linked neuroinflammation in AD (Gonzalez-Reyes et al. 2017). Several reports already point towards an interaction between reactive astrocytes and peripheral immune cells (Escartin et al. 2019). Treg cells attenuate the A1-like phenotype of reactive astrocytes by regulating STAT3, IL-6, and amphiregulin signaling (Ito et al. 2019). However, STAT3-positive reactive astrocyte subpopulation (indicating the A2-like subtype) can regulate the innate and acquired immune system by restricting the recruitment of CD8+ lymphocytes while increasing the population of CD74+ microglia/macrophage thus inducing a pro-metastatic environment (Priego et al. 2018). Thus, such complex and bidirectional communication between reactive astrocytes with various types of peripheral immune cells remains to be investigated in AD models where evidence of a leaky BBB abounds.

Microglia-Astrocyte Cross Talk

It is often considered that microglia are the first ones to respond to an injury or disease through cytokine secretion. The activated microglia then further instigate the astrocytes towards their reactive profiles (Sofroniew 2014b). It is already established that upon LPS injection, activated microglia secrete IL1α, TNFα, and C1q to induce the A1 astrocytes (Liddelow et al. 2017). However, methotrexate chemotherapy can activate microglia which in turn trigger the A2-like phenotype of astrocytes (Gibson et al. 2019). Reports indicating that IL-10 exacerbates Aβ-associated phenotypes in mouse models of AD suggested that the microglial IL-10 triggered astrocytic ApoE4 expression which then acting in a loop reduced the ability of microglia to phagocytose Aβ (Chakrabarty et al. 2015; Guillot-Sestier et al. 2015). Hence, it seems that the microglia dictate the reactive astrocyte phenotypes, but the reverse may also be true. Evidence indicates that reactive astrocytes can also upregulate the release of various cytokines and chemokine molecules that can trigger the activated profiles of the neighboring microglia (Ceyzeriat et al. 2018; Orre et al. 2014a; Zamanian et al. 2012). For example, direct Aβ-induced NF-κB pathway activation in reactive astrocytes eventually secrete an enhanced level of C3 extracellularly, which can then induce the expression of C3a receptor on both microglia and neuron further leading to an impaired microglial Aβ uptake and eventual plaque formation and cognitive damage in AD (Lian et al. 2016).

Astrocytic Neuroinflammatory Profile Correlates with AD Stage

Astrocyte and microglia—the major responsive cells themselves behave differently depending on the context and extent of neuroinflammation. Hence, their own phenotype as well as their secretion profiles may reflect their contribution towards the neuroinflammatory changes. Transgenic models with cell type-specific loss of functions have been used to study intracellular effector molecules that dictate the astrocytes' pro-inflammatory or anti-inflammatory functions. Some of the molecules, including SOCS3, NF-κB, ACT1, CCL2, CXCL10, and VEGF, promote astrocytic proinflammatory reactions in response to CNS injury and inflammation. The anti-inflammatory role of astrocytes is mediated by several cytokines like TGF-β, IL-6, IL-10, IL-11, IL-19, and IL-27 and intracellular molecules like STAT-3, A20, GAL9, and CRYAB. It has also been suggested that the expression of several receptors like ER-α, GP-130, and DRD-2 exert their effect in diminishing the acute pro-inflammation (Sofroniew 2015). Recent reports also suggest that small intercellular effector molecules like retinoic acid not only reduce neuroinflammation and oxidative stress but also prevent BBB damage (Mizee et al. 2014). Few miRNAs like miR-181, miR-17-5p, and associated proteins, namely, Dicer-1, have been reported to get activated during neuroinflammation which may be needed to initiate the scar forming proliferating astrocytes through activating JAK-STAT signaling mechanism (Hutchison et al. 2013; Hong et al. 2014).

While Pekny et al. have shown the mechanism of reactive astrogliosis as a defensive phenomenon undertaken by astrocytes at initiation of the disease (Pekny et al. 2016), chronic Aβ presence may eventually activate astrocytic cell receptors

such as receptor for advanced glycation end products (RAGE) which in turn induces the NF-κB pathway. Proinflammatory cytokine IL-1β, thus secreted downstream of the pathway, act on astrocytes in an autocrine manner inducing further Aβ synthesis and enhances astrocytes' pro-inflammatory secretion profile (Gonzalez-Reyes et al. 2017). Thus reactive astrogliosis conventionally observed as an inflammatory response in AD brain may be alternatively seen as a highly complex, diverse, and multistep phenomenon with both detrimental and protective facets depending on context such as the stage in AD progression. Heneka et al. (2015) have already reported a transition of microglia from its beneficial M2 phenotype to the detrimental M1 phenotype represented by their characteristic cytokine profiles from an inflammatory perspective during disease progression. A similar shift may be predicted in case of astrocytes, a significant partner in "neuroinflammation" crime at later stages of the disease. Thus, neuroinflammation is not necessarily an outcome of amyloid plaque deposition in AD but plays a greater role in modulating disease progression.

2.4 Role of Astrocytes in Aβ Clearance and Production

Aβ Uptake and Clearance

The very first indication that astrocytes may play a role in Aβ clearance was reported by Wyss Corey et al. (2003) in cultured mouse astrocytes which have the ability to uptake and degrade Aβ. Aβ-containing astrocytes were also detected earlier by Funato et al. (1998) in an aged human brain, by Thal et al. in human entorhinal cortex (Thal et al. 2000), and by others in AD brain especially in those surrounding amyloid plaques (Kurt et al. 1999; Yamaguchi et al. 1998). The astrocytes surrounding Aβ plaques are mostly recognized as reactive astrocytes which not only restrict the Aβ plaques from diffusing outside, thus limiting their growth and preventing them from affecting the healthy neurons (Sofroniew 2009) but also are involved in increased Aβ phagocytosis (Arranz and De Strooper 2019). In fact both ex vivo and in vivo studies using adult human or mouse astrocytes transplanted in plaque-bearing AD animal models re-established that astrocytes indeed phagocytose and clear Aβ (Koistinaho et al. 2004; Pihlaja et al. 2008; Nielsen et al. 2009, 2010). Consistently, ablation of GFAP or vimentin-expressing reactive astrocytes lead to an increase in plaque growth in APP/PS1 mice (Kraft et al. 2013). Importantly, almost all isoforms of Aβ such as monomers, oligomers, and fibrils can be found within astrocytes in cortices of AD patients (Osborn et al. 2016). Basically two mechanisms are at play in reactive astrocyte-mediated Aβ plaque removal—phagocytosis (uptake followed by lysosomal degradation) and secretion of various Aβ-degrading enzymes (Perez-Nievas and Serrano-Pozo 2018).

Astrocytes have several receptors that can mediate Aβ uptake. However, whether the uptake will subsequently lead to degradation and clearance of Aβ or will result in accumulation and destruction of the astrocytes themselves depends on multiple factors including age or stage of the disease, relative expression levels of various receptors, receptor isoforms, or intracellular signaling changes following uptake. Low-density lipoprotein receptor-related protein 1 (LRP1) is one such receptor that

is responsible for uptake and clearance of Aβ (Basak et al. 2012; Kim et al. 2009). But it is also recognized as a receptor of ApoE and ApoE-Aβ complexes where ApoE itself is considered a major contributor in Aβ removal (Garwood et al. 2017). Reports suggest that both ApoE-dependent as well as ApoE-independent mechanisms exist for Aβ uptake. ApoE-null astrocytes are found to be less effective in Aβ plaque clearance (Koistinaho et al. 2004) which contradicts another study that showed Aβ and ApoE compete between themselves for binding with LRP1 on astrocytes without interacting with each other (Verghese et al. 2013). Moreover, ApoE is found in reactive astrocytes surrounding the Aβ plaques (Terai et al. 2001; Koistinaho et al. 2004). However, ApoE is also linked to the initial seeding of Aβ plaques (this will be discussed later in this section). Besides LRP-1 and ApoE, low-density lipoprotein receptor expressed mostly in astrocytes can also uptake Aβ in an ApoE-independent manner (Basak et al. 2012; Katsouri and Georgopoulos 2011). An enhanced expression of scavenger receptor class B member 1 (SRB1) was detected in astrocytes in response to Aβ especially when Aβ was associated with either serum amyloid P-complement component 1, q subcomponent (SAP-C1q), or ApoE (Mulder et al. 2012). Earlier it was already reported that SRB1 is linked with Aβ uptake in adult mouse as well as in human astrocytes (Wyss-Coray et al. 2003; Husemann and Silverstein 2001). Moreover, there is evidence of Aβ binding and engulfment via CD36 and CD47 receptors in cultured astrocytes (Jones et al. 2013).

Astrocytes play another significant role in initial stages of AD by removing and subsequently degrading Aβ from brain parenchyma across BBB draining in the perivascular space (Cai et al. 2017). Three main receptors of Aβ that come into play here are—aquaporin-4 (AQ4) mediates perivascular drainage of Aβ from CSF (Iliff et al. 2012), and LRP1 regulates the efflux of Aβ from the brain into circulation while another receptor RAGE regulates the influx of Aβ from the circulation into brain (Cai et al. 2017). Relative LRP1 and RAGE receptor expression are a crucial factor in AD progression. Following Aβ uptake, several changes may occur within astrocytes. Tissue samples from patients with AD showed an upregulated level of a chaperone complex called heat shock protein 8-Bcl2-associated anthanogene 3 (linked to misfolded protein degradation) in astrocytes and has a plausible role in cytoskeletal remodeling in areas of neuronal damage (Seidel et al. 2012). The astrocyte-mediated phagocytosed Aβ is suspected to be degraded via lysosomal degradation at least at the early stages of disease progression. Increasing lysosomal biogenesis within astrocytes in APP/PS1 mice by introduction of transcription factor EB resulted in enhanced Aβ phagocytosis and breakdown (Xiao et al. 2014). Reactive astrocytes, especially at the early stages of AD, may also influence the surrounding microglia to increase their Aβ phagocytosis ability (Kim et al. 2018). However, in the later stages of AD, microglia may be actively induced by reactive astrocytes to contribute to Aβ deposition.

Postmortem studies on AD patients have also revealed several Aβ-degrading enzymes expressed by reactive astrocytes, namely, insulin degrading enzyme (IDE), neprilysin (NEP), endothelin-converting enzyme-2 (ECE-2), and matrix metalloproteinases (MMPs) (Carter et al. 2019). IDE expression is enhanced in reactive astrocytes surrounding amyloid plaques in Tg2576 mouse (refer to

Table 1 for details) (Leal et al. 2006). Metalloprotease NEP can be secreted by reactive astrocytes as well as microglia for extracellular Aβ degradation (Mohajeri et al. 2002; Apelt et al. 2003). ECE-2 on the other hand is released by reactive astrocytes as well as by neurons and microglia for Aβ breakdown (Palmer et al. 2009). MMPs such as MMP-2 and MMP-9 also show increased expressions in reactive astrocytes around Aβ plaques such that inhibitors of MMP-2/9 reduce astrocyte-conditioned medium-mediated Aβ degradation (Yin et al. 2006). However, this MMP concept contradicts our work (Saha et al. 2020) which shows that TIMP-1 induces Aβ plaque clearance from AD rat brain. The contradiction may be due to difference in experimental approaches or may be due to the binding of TIMP-1 to other receptors rather than MMPs to show a neuroprotective role. Nevertheless, other mechanisms involving ApoJ, α2-macroglobulin, α1–21 antichymotrypsin, and secretion from astrocytes also assist Aβ degradation (Carter et al. 2019). They can even bind to Aβ attenuating their ability to constitute insoluble plaques subsequently facilitating their clearance across BBB (Ries and Sastre 2016).

Aβ Production and Astrocytes

Reactive astrocytes are also involved in Aβ production. Aβ itself can upregulate the astrocytic expressions of BACE1, APP, and the processing of β-secretase and γ-secretase enzymes resulting an increased accumulation of oligomeric Aβ that develops further into amyloid plaques (Garwood et al. 2017; Grolla et al. 2013a, b). For example, BACE1 primarily expressed in neurons for amyloidogenic cleavage of APP is also detected within reactive astrocytes encompassing the Aβ plaques in transgenic models of AD (Jin et al. 2012; Orre et al. 2014b; Yamamoto et al. 2007) and in patients with AD (Hartlage-Rubsamen et al. 2003; Rossner et al. 2005). Aβ may also activate astrocytes into secreting various cytokines that are responsible for increasing the amyloidogenic cleavage of APP in astrocytes (Garwood et al. 2017). A cytokine TNFα was linked to the enhanced expression of astrocytic BACE1 and the resultant Aβ deposition (Yamamoto et al. 2007). Notably, other reports supported the finding that TNFα, IFNγ, and Aβ itself can enhance the expression of BACE1, APP, and production of Aβ from astrocytes in vitro and in vivo (Zhao et al. 2011). Indeed LPS injection in mouse (now denoted as the A1-like astrocyte model) reportedly increased the APP expression by twofold in an APPswe mouse brain (refer to Table 1 for details) (Liddelow et al. 2017; Sheng et al. 2003). Consistently, LPS injection induced a magnified BACE1 activity and a threefold enhancement in both Aβ42 and Aβ40 production with a concurrent upregulation in GFAP expression in the cortex and hippocampus (Sheng et al. 2003). Interestingly, energy deficiency especially the hypometabolic state in advanced AD stages increases BACE1 expression and amyloidogenic processing of APP in astrocytes producing Aβ both in vitro and in vivo (Sheng et al. 2003). Both amyloidogenic and non-amyloidogenic processing of APP result in APP intracellular domain (AICD) which negatively regulates LRP1 expression. In reactive astrocytes associated with amyloid plaques, AICD levels are high and hence may be implicated in inhibition of astrocytic LRP1-mediated Aβ phagocytosis (Osborn et al. 2016). In contrast to the concept of AQ4-mediated Aβ clearance from CSF,

upregulated levels of AQ4 in plaque-surrounding reactive astrocytes in Tg-ArcSwe AD mouse model (refer to Table 1 for details) may also suggest a contribution of this protein in plaque formation (Yang et al. 2017).

A Hypothesis for Astrocyte Function in Aβ Clearance or Its Production

Overall, the reactive astrocytes in AD may either induce Aβ uptake and clearance, or they may accelerate Aβ production albeit under disparate circumstances that are elaborated below. "Age" is a major factor that decides the ability of astrocytes in uptaking and subsequently degrading Aβ in an effective manner in AD (Garwood et al. 2017). The intracellular presence of Aβ in astrocytes often interpreted as a neuroprotective phenomenon leading to Aβ clearance may be contrarily implicated in Aβ plaque progression. Inability in degrading Aβ following uptake especially at advanced AD stages may lead to the death of Aβ-overloaded astrocytes and further contribute to secondary plaques (Nagele et al. 2004). It was also indicated that uptake of Aβ may subsequently alter the astrocyte's phenotype (Garwood et al. 2017), but whether it would proceed from an initial protective A2-like phenotype to the later detrimental A1-like upon Aβ uptake is not yet known. Allaman et al. (2010) reported that astrocytic Aβ uptake via type A scavenger receptor family altered the signaling pathway that regulated astrocytic metabolism inciting detrimental effect on nearby neurons, corroborating the idea of Aβ uptake not necessarily being beneficial. All the mechanisms of Aβ clearance may be progressively challenged in AD. Astrocytes of older AD mouse internalized 20% and 35% lower amount of Aβ compared to those from a younger AD mouse in vitro and in vivo, respectively (Iram et al. 2016), strongly establishing the reduced ability of Aβ uptake in later stages of AD. It was further observed that the older AD mouse astrocytes had a diminished expression of SRB1 compared to that of the younger ones leading to the reduced Aβ uptake (Iram et al. 2016). In advanced AD stage, after Aβ uptake, the Aβ protofibrils may not be degraded rather they can lead to the formation of large astrocytic endosomes (Sollvander et al. 2016) resulting in defective astrocytic degradation pathway (Acosta et al. 2017). Interestingly, the Aβ-degrading enzymes are also dysregulated in astrocytes especially in advanced stage of AD. For example, NEP shows an overall reduced expression in the hippocampal and cortical regions in aged Tg2576 mice as well as in post mortem tissues from AD brain although its expression remained upregulated in reactive astrocytes near the Aβ plaques (Carter et al. 2019). Astrocyte dysfunction seems to be a consequence of AD progression, but is it really a dysfunction or an altered phenotype of astrocyte needs investigation. Metabolic alteration of astrocytes can upregulate RAGE and attenuate the expression of LRP1 receptors further enhancing RAGE-mediated Aβ uptake, subsequent NF-κB pathway activation upregulating pro-inflammatory cytokine secretion, and production of C3. C3 reduces Aβ phagocytosis (Zulfiqar et al. 2019).

Altogether, it seems that the initial activation of astrocytes is protective with increased expression of Aβ receptors in astrocytes around Aβ plaques, increased Aβ phagocytosis, upregulated lysosome-mediated Aβ degradation, as well as enhanced expression of Aβ-degrading enzymes. Hence, these rapidly reactivated astrocytes in AD may reflect the protective A2-like phenotype. This stage may also be identified

with protective ApoE2 isoform expressing astrocytes. With the gradual disease progression if astrocytes become overactivated, their secretion profile turns neurotoxic, leading to dramatic metabolic alteration, increased amyloidogenic processing of APP, suppressing microglia-mediated Aβ phagocytosis, and ApoE becoming neurotoxic expressing majorly ApoE4 isoform (Kim et al. 2018). ApoE4 is associated with impaired Aβ uptake by astrocytes compared to ApoE3 astrocytes. Diminished Aβ uptake in ApoE4 astrocytes has been linked with excessive endosomal acidification and impaired autophagy. It is also thought to contribute greatly to the initial seeding of Aβ plaques (Arranz and De Strooper 2019). Thus, depending on AD progression and altering reactive astrocyte phenotypes, expression of different ApoE isoforms may also vary.

According to a recent report which showed a kinetic alteration from initial beneficial to later detrimental reactive astrocyte phenotype in AD model in vitro, it may be hypothesized that a younger AD mice may be expressing more of A2-like reactive astrocytes associated with upregulated Aβ clearance while the older AD mice may have a higher population of A1-like astrocytes contributing towards plaque progression (Saha et al. 2020). The duration of Aβ existence can itself decide the fate of astrocytic phenotype and in turn its role in Aβ clearance or production. A report has indicated that Aβ rapidly activates astrocyte and microglia to release TGFβ which further upregulated microglial Aβ uptake, protecting neurons from Aβ toxicity and thus may resemble the A2-like astrocyte (Arranz and De Strooper 2019). In contrast, chronic presence of Aβ can lead to enhanced C3 expression through NF-κB pathway activation, which can bind to microglia reducing its ability to phagocytose Aβ (Arranz and De Strooper 2019). Thus, this astrocyte phenotype is not necessarily a malfunction but may characterize the detrimental A1-like phenotype at the later stages of AD leading to the development of Aβ pathology through a vicious cycle of astrocyte pathogenesis and Aβ pathology.

2.5 Reactive Astrocytes in Tau Pathology

Abnormal levels of phosphorylation in tau, a microtubule-associated protein, lead to the generation of helical filaments along the length of axons in neurons. They are commonly termed as neurofibrillary tangles (NFT) and are recognized as a major pathological species in AD besides Aβ. In AD, following the aberrant levels of hyperphosphorylation, tau's affinity to the microtubule is diminished, and hence, free tau level is enhanced leading to their accumulation and subsequent aggregation. The consequence of these events results in the formation of glial and neuronal tangles in dystrophic neuritis. Importantly, tau pathology directly contributes to disease progression by inducing neurodegeneration and correlates well with degree of cognitive impairments in AD (Ballatore et al. 2007).

In addition to their well-established association with amyloid pathology, reactive astrocytes increase in the vicinity of NFTs. Using postmortem human AD brain samples, it has been shown that astrogliosis occurs near amyloid plaques as well as around the NFTs and its level is enhanced linearly during the disease progression

even when amyloid burden is no longer increasing. Intriguingly, astrogliosis and microgliosis correlate positively with the NFT burden throughout the clinical phase in AD (Serrano-Pozo et al. 2011). Appearance of tau pathology within astrocytes is different across various tauopathies including AD. Glial reactivity is more strongly associated with thorn-shaped astrocytes, detected in AD, Pick's disease, supranuclear palsy, and featuring perinuclear deposits of tau, compared to other types of astrocytes. Moreover, the pathologic tau species modulates glial functions in a way that is detrimental to the neurons and glia themselves (Kahlson and Colodner 2015).

Glial reactivity and neuroinflammation are often considered as secondary outcomes of tauopathies. In an AD mouse model featuring pathologic forms of both Aβ and tau, blocking IL-1β signaling altered inflammatory reactions in the brain, ameliorated cognitive functions, significantly reduced tau pathology and diminished the levels of astrocyte-derived s100β (Kitazawa et al. 2011). Furthermore, a novel risk gene for AD, triggering receptor expressed on myeloid cells 2 (TREM2, uniquely expressed by microglia) is strongly associated with tau pathology. In an animal model of tauopathy, microglia regulate astrocyte reactivity in a TREM2-dependent manner (Leyns et al. 2017). Similarly, ApoE4 affects tau pathology as well as dictates the ensuing neurodegeneration in an Aβ independent manner in tauopathic mouse (Shi et al. 2017). Therefore, these findings suggest that TREM2 and ApoE4 are the major inducers of neuroinflammation leading to accelerated tau pathology and neurodegeneration independent of Aβ pathology. Further investigations are required to fully understand the role of TREM2 and ApoE4 on the development of tau pathology.

2.6 Role of Astrocytes in Modulating Synaptic Plasticity in AD

Physiological Role in Synaptic Health

The bidirectional exchange between astrocyte and neuron begins with specific neurotransmitters being released from the presynaptic cleft which triggers specific metabotropic or ionotropic receptors on the astrocytic membrane upon binding as discussed earlier (Garwood et al. 2017). This activation can be further communicated to the surrounding astrocytes via gap junctions or through the release of a range of secretory molecules (Porter and McCarthy 1996; Giaume et al. 2010)—glutamate, GABA, D-serine, ATP, etc.—which can regulate synaptic plasticity by modulating LTP and LTD (Singh and Abraham 2017). Astrocytes also release cytokines that may influence synaptic plasticity in a healthy brain albeit at low level of secretion including TNF-α, IL-6, IL-2, IL-1β, IL-10, IFN-γ, and IFN-α (Habbas et al. 2015; Singh and Abraham 2017).

Reactive Astrocytes in Synaptic Dysfunction in AD

Synaptic dysfunction occurs during the prodromal stage in AD that progressively leads to memory impairment. LTP, LTD, as well as synaptic scaling all display aberrations in AD models (Selkoe 2008; Cheng et al. 2009; Rowan et al. 2014).

Abnormal and sporadic calcium signaling is observed in astrocytes in a transgenic animal model of AD (Kuchibhotla et al. 2009; Takano et al. 2006) as well as in astrocyte culture treated with Aβ peptide (Haughey and Mattson 2003). This aberrant Ca2+ signaling, which is initiated near the Aβ plaques, often spreads to longer distances across the cortex indicating that the abnormal Ca2+ signaling in astrocytes induces a sprawling effect. This abnormal and hyperactive Ca2+ signaling is coincident with reactive astrocytes surrounding the plaques while atrophic astrocytes are present at a distance from the plaques (Rodriguez et al. 2009). Atrophic astrocytes further exacerbate synaptic dysfunction due to their reduced ability to contact synapses.

Besides, there is GABA which displays an elevated level in AD patient's CSF (Samakashvili et al. 2011), and thus antagonists for GABA receptor improved LTP and memory in AD mouse models (Yoshiike et al. 2008). This GABA is majorly contributed by reactive astrocytes with higher monoaminoxidase-B (MAO-B) and reduced glutamine synthetase (GS) expressions (Acosta et al. 2017). Subsequently, the tonic GABAergic influence, low under normal conditions, gets abnormally upregulated in mouse AD models and human AD especially around the Aβ plaques (Jo et al. 2014; Wu et al. 2014). Reactive astrocyte-associated GABA upon secretion initiates inhibition of excitatory neurotransmission by dramatically diminishing the probability of neuronal secretion and thus inhibits LTP initiation. LTP and memory impairments observed in AD patients were diminished by inhibition of astrocytic GABA synthesis or release (Acosta et al. 2017), thus underscoring the importance of studying reactive astrocyte-associated GABA in greater detail in AD pathogenesis. In contrast, Aβ-induced ATP released from astrocytes protected neurons against Aβ-induced LTP dysfunction in culture (Jung et al. 2012). Thus, this may be a part of defense mechanism undertaken by reactive astrocytes early in AD progression, protecting learning and memory function. TNF-α on the other hand, in AD, is released from reactive astrocytes at levels dramatically higher than physiological levels in presence of Aβ (Johnstone et al. 1999) and its silencing reduced amyloid-dependent LTP suppression (Wang et al. 2005) and memory dysfunction (Alkam et al. 2008). Thus, TNF-α can cause aberrant long-lasting upregulation in synaptic strength (Habbas et al. 2015). Reactive astrocytes in AD seems to follow the A1-like astrocytic fate (Liddelow et al. 2017; Shi et al. 2017), at least later in disease progression, secreting pro-inflammatory cytokines such as IL-6, IL-1β, IFN-γ, as well as TNF-α (Benzing et al. 1999; McGeer and McGeer 2010) that are potent inhibitors of LTP, at levels higher than the normal (Singh and Abraham 2017). Astrocytic glutamate and D-serine, co-agonists of NMDAR, also display aberrant behavior in AD and can be attributed to reactive astrogliosis. NMDAR-dependent synaptic plasticity regulation is damaged in mouse AD models and human AD (Battaglia et al. 2007) possibly due to aberrant glutamate accumulation in the synaptic cleft, abnormal activation of extrasynaptic NMDAR (Li et al. 2011c) and elevated D-serine levels (Madeira et al. 2015). Thus, a complex alteration of reactive astrocyte functions in AD displayed through diverse temporal profiles of secreted molecules, characteristic of astrogliosis, can be correlated to its contribution from

protecting synapse to inducing LTP deficits and the accompanied memory impairment.

2.7 Role of Astrocytes in Neurotransmitter Recycling in AD

Astrocytes in Glutamate Regulation in AD

Glutamate-induced neurotoxicity is associated with neuron death and eventually in disease progression in AD (Lipton 2004). An excess of glutamate in the synapse leads to neuronal death through a series of toxic events including a large amount of Ca2+ influx into neurons activating endonucleases, phospholipases, and proteases further leading to cellular structure damages and is collectively termed as glutamate excitotoxicity (Manev et al. 1989). Astrocytes uptake 80% of the glutamate from the synaptic cleft in comparison to 20% by postsynaptic dendritic transporters (Acosta et al. 2017). As already mentioned, EAAT2, one of the glutamate transporters in human astrocytes, displays an attenuated expression in human AD brain and in APP transgenic mice (Woltjer et al. 2010; Schallier et al. 2011) while both EAAT1 and EAAT2 showed diminished expressions in cultured astrocytes treated with Aβ42 (de Vivo et al. 2010; Matos et al. 2012). Introduction of ceftriaxone to upregulate EAAT2 levels in 3xTg-AD model (refer to Table 1 for details) ameliorated their cognitive deficits (Zumkehr et al. 2015) and is being developed as a drug for treating glutamate excitotoxicity in diseases involving motor neurons (Ji et al. 2005). Moreover, astrocytic EAAT1 ablation leads to neuronal excitotoxicity and tau pathology (Kilian et al. 2017).

In rodent models of AD, GLT-1 and GLAST expressions were reduced both at the protein and mRNA levels (Garcia-Esparcia et al. 2018; Peters et al. 2009). Since, GLT-1 is responsible for 90% of the astrocytic glutamate uptake in the brain, its deficit accelerated memory impairment upon crossing a GLT+/− mouse with an APP/PS1 mouse (Mookherjee et al. 2011). Reports have suggested that Aβ hinders the uptake of glutamate in astrocytic cultures (Harkany et al. 2000; Fernandez-Tome et al. 2004) resulting in glutamate excitotoxicity. The probable reason is the reduced expression of GLT-1 associated with Aβ depositions (Masliah et al. 1996; Huang et al. 2018), for example, a reduction in GLT-1 expression was detected in the proximity of amyloid plaques in APP/PS1 mouse (Hefendehl et al. 2016) or Aβ induced GLT-1 internalization from the astrocytic membrane (Scimemi et al. 2013). Moreover, GLT-1 expression was decreased in the frontal cortex of postmortem AD brain and was correlated with reduced D-[3H]-aspartate binding to the brain tissue, a measurement for glutamate transporter activity (Li et al. 1997; Anderson et al. 2001). This reduction in binding of D-[3H]-aspartate in the AD brain frontal cortex was also linked with decreased expression of synaptophysin, a marker for synaptic health, thus indicating that impaired transport of glutamate may lead to glutamate excitotoxicity and related neurodegeneration including synaptic damage (Masliah et al. 1996). Additionally, splice variants of GLT-1 localized in the glial processes surrounding the plaques (Pow and Cook 2009) were found to be the inactive variants and play a dominant-negative role by inhibiting normal glutamate uptake (Scott et al.

2011). It was found that this splice variant of GLT-1 has a high expression especially in regions affected in neurodegeneration, associated with a large number of reactive astrocytes. Thus increase in reactive astrocytes in AD-vulnerable areas of the brain with decreased capacity for uptake of glutamate may lead to glutamate excitotoxicity (Acosta et al. 2017).

Similarly, Aβ also attenuated GLAST expression and GLAST-dependent glutamate uptake in cortical astrocyte culture model of AD (Jacob et al. 2007; Matos et al. 2008). However, few reports have also indicated Aβ associated upregulation in astrocytic glutamate uptake via GLAST alongside an increased GLAST expression (Ikegaya et al. 2002; Abe and Misawa 2003). These contradictory outcomes may be the result of variations in Aβ preparation methodologies as well as culture conditions (Acosta et al. 2017). A regional difference in Aβ associated GLAST expression was observed as GLAST expression increased in the hippocampus and dentate gyrus of AD brain while it displayed a decreased expression in the cerebellum (Jacob et al. 2007).

Aβ can also induce glutamate release from activated astrocytes via alpha-7nAch receptor (Acosta et al. 2017), activating extrasynaptic NMDA receptors leading to Aβ-dependent synaptic depression and loss of spines (Talantova et al. 2013). A-β-dependent release of glutamate from reactive astrocytes may also take place via connexin hemichannel that can induce neuronal death (Orellana et al. 2011). Thus, a dysregulation in glutamate uptake alongside its release from astrocytes activated by Aβ increases the lifetime of glutamate in the synapse culminating in Aβ-induced excitotoxicity in AD.

Astrocytes in GABA Regulation in AD

GABA is taken up in astrocytes by dedicated GAT-1 and GAT-3 transporters and gets catabolized by GABA-transaminase (GABA-T) to succinate and enters the TCA cycle to produce alpha ketoglutarate, glutamate, and glutamine besides ATP (Schousboe et al. 2017; Walls et al. 2015). Nontoxic glutamine is then extruded by specialized transporters into the extracellular space from where it is imported by glutamatergic and GABAergic neurons to be used as a precursor for the synthesis of glutamate and GABA, respectively, as a part of glutamine-glutamate-GABA cycle in healthy individuals (Pow and Crook 1996; Eid et al. 2012).

Due to this energy driven breakdown of GABA in astrocytes, the GABA concentration in the astrocytes remains low in a healthy young brain (Verkhratsky et al. 2019a). However, a dysregulation in GABA metabolism is observed in astrocytes with aging and in neurodegeneration. The concentration of GABA is significantly elevated in AD patients and in transgenic mouse model of AD and was traced to the reactive astrocytes near the Aβ plaques (Jo et al. 2014; Wu et al. 2014; Brawek et al. 2018; Acosta et al. 2017). These alterations in GABA concentration in astrocytes is correlated to a concomitant upregulation in the expression of glutamic acid decarboxylase (GAD67), a GABA-producing enzyme, alongside an upregulation in astroglial MAO-B, an enzyme that synthesizes GABA from putrescine, a type of polyamine (Jo et al. 2014). This phenomenon is accompanied by a down-regulated expression of GS in reactive astrocytes near the amyloid plaques in the prefrontal

cortex and hippocampus of 3xTg-AD mice (Olabarria et al. 2011). Thus, the reactive astrocytes start synthesizing GABA, either utilizing glutamate, with its increased availability due to reduced expression of GS and increased expression of GAD67 or via the MAO-B pathway (Verkhratsky et al. 2019a). Astroglial GABA thus synthesized is released from astrocytes via Bestrophin-1 Cl-channels or via reversed GAT3 transporters. GABA, released into the extracellular space, may either be seen as a mediator in impaired neuronal activity and memory loss in AD mice (Jo et al. 2014), or it can be seen as a defensive mechanism to hinder the neuronal hyperexcitability, characteristic of AD, with an increased tonic inhibition (Verkhratsky et al. 2019a). Hence, though there is a controversy about the role of GABA being protective or detrimental, one can conceive the plausible idea that the upregulated GABA, rather, plays a protective role towards neurons against its over-excitation at the cost of cognitive deficits (Chun and Lee 2018).

2.8 Role of Astrocytes in Neuron Death and Survival in AD

As discussed above, most of the astrocyte's normal functions are altered in AD. This includes metabolic activity (Escartin et al. 2019), gliotransmitter release (Jo et al. 2014), neurotransmitter uptake (Escartin et al. 2007; Sheldon and Robinson 2007), release of a plethora of cytokines and chemokines (Sofroniew 2014b), and phagocytosis (Gomez-Arboledas et al. 2018). Hence, these astrocytes with a range of functional alterations indicate varying degree of reactivity attributed to multiple factors including disease stage, eventually translated to their role as beneficial or detrimental partners for neurons.

Reactive Astrocytes Mediating Neuron Death

Interestingly, most of the genes associated with the risk of developing AD are expressed in glial cells, mostly in astrocytes, namely, clusterin (ApoJ), sortilin-related receptor 1, fermitin family member 2, and most importantly ApoE (Arranz and De Strooper 2019). Postmortem studies on human AD brains using laser capture microdissection (LCM) have revealed the transcriptomic alterations that specifically take place in astrocytes when compared to healthy controls. Using GFAP as the astrocyte marker for LCM, transcriptomic profile of activated astrocytes greatly contrasted between advanced Braak stage of NFTs (V-VI) representing an advanced AD phenotype, versus low Braak stages (I–II). Aberrations in genes related to proliferation, apoptosis, actin cytoskeleton, and ubiquitin-dependent proteolysis were observed at the low Braak stages that is highly in contradiction with the altered gene expression profile observed in intracellular signaling pathways like PI3K/AKT, insulin, and MAPK pathways at advanced stages of AD pathogenesis (Simpson et al. 2011). Another known astrocyte marker, aldehyde dehydrogenase 1 family member L1 (ALDH1L1), was used in an independent study by LCM that revealed an elevated expression in genes associated with astrocytic immune reaction and mitochondrial activity especially in posterior cingulate gyrus where Aβ deposition was prominent in contrast to healthy controls (Sekar et al. 2015). Concomitantly, Orre

et al. (2014a) observed that in astrocytes stringently isolated from APP/PS1, AD mice develop prominent amyloid plaques that are similar to AD plaques found in humans, expression of genes associated with the neuronal support mechanism and playing a crucial role in neuronal communication were compromised and that elicited a pro-inflammatory response promoting neuronal death (Orre et al. 2014a). Notably, the A1 astrocytes through the complement system also play a significant role in inducing deterioration in neuronal health (Liddelow et al. 2017).

Reactive Astrocytes in Neuron Survival

Accumulating evidences also indicate towards a role of astrocytes in protecting neurons in AD. As discussed earlier, Wyss-Corey et al. and others established that astrocytes can phagocytose and eventually degrade Aβ (Wyss-Coray et al. 2003; Funato et al. 1998; Thal et al. 2000). Garwood et al. raised a crucial question whether age can be a pivotal factor in determining the efficiency of this astrocytic ability (Garwood et al. 2017), in other words, whether age can direct the astrocytic fate in AD. Postmortem references only capture snapshots of a bigger and more complex astrocytic dynamicity in AD, and in vitro models are limited depending on the experimental conditions not indicating temporal heterogeneity in astrocyte type and/or profile and their subsequent effects on neurons. Hence, it is worth considering that with aging or slow disease progression in AD, astrocytes may progressively lose their ability to effectively degrade Aβ upon its uptake and thus a change in their phenotype may be observed eventually inducing plaque progression (Garwood et al. 2017).

Sofroniew and colleagues have repeatedly tried to establish that reactive astrocytes constitute a scar-like barrier that can restrain the damaged tissues from affecting the surrounding neurons and can stimulate CNS axonal regrowth (Sofroniew 2009, 2015). An astrocytic scar creates a physical barrier between Aβ plaques and the neuropil surrounding them. This may restrict the collateral damage that diffusible and soluble toxic Aβ oligomer can confer on the nearby neurons by detaching them from the plaques (Liddelow et al. 2017; Perez-Nievas and Serrano-Pozo 2018). Neuroprotective ability of reactive astrocytes could be further attributed to their increased ability to phagocytose plaque-associated damaged neurites in APP/PS1 mice as well as in AD patients (Gomez-Arboledas et al. 2018). These beneficial astrocytes may very well represent the A2-like subtype of reactive astrocytes. A2-like astrocyte can be identified with a characteristic transcriptome with an upregulated expression of neurotrophic genes, namely, S100 calcium-binding protein a10, cardiotrophin-like cytokine factor 1, pentraxin 3, transglutaminase 1, or sphingosine kinase 1. A2-like astrocytes thus release a series of beneficial factors that promote neuron survival and growth and secrete thrombospondins associated with repair of damaged synapses. Liddelow and Barres (Liddelow et al. 2017; Liddelow and Barres 2017) also indicated that STAT-3 may be an upstream activator of A2 astrocytes and JAK-STAT3 pathway may be mediating this activation. This pathway is involved in regulating several cellular functions such as cell proliferation, cell differentiation, growth, etc. Their proposal can be substantiated by an earlier report where STAT3-dependent reactive astrocytes diminished axotomized

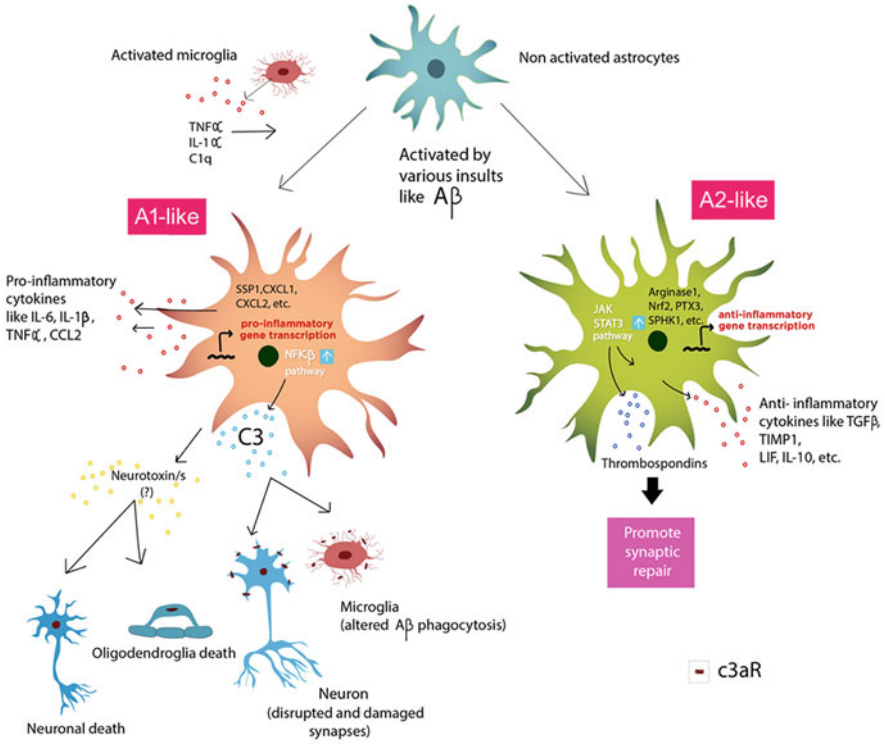

Fig. 2 Schematic representation of astrocyte reactivation in Alzheimer's disease: An astrocyte may be activated by various insults such as Aβ in Alzheimer's disease and may give rise to different reactive astrocyte subtypes including A1-like and A2-like, in a disease-stage specific or age-related manner. However such a concept is speculative and will require further research. Neurotoxic reactive astrocyte A1-like and neuroprotective A2-like have distinct transcriptomic profiles as indicated. A1 phenotype can be alternatively activated by activated microglia through the secretion of TNFα, IL-1α, and C1q and secretes a yet-to-be identified neurotoxin that induces neuron and oligodendroglia death. NF-κB pathway is upregulated in A1 leading to the release of complement protein C3 in the extracellular space which can further bind to C3aR receptors on neurons and microglia deteriorating synaptic health and ability of microglia to phagocytose Aβ. A1-like also releases several pro-inflammatory cytokines as indicated. A2-like conversely shows upregulated expression and release of several anti-inflammatory cytokines as indicated and thrombospondins that can promote synaptic repair. JAK/STAT3 pathway is upregulated in A2-like astrocytes (Liddelow and Barres 2017)

motoneuron degeneration in the facial motor nucleus and promoted synaptic repair through thrombospondin release (Tyzack et al. 2014). Altogether, the above studies allude to the presence of A2-like astrocytes at the prodromal stage of AD probably being activated by JAK-STAT3 pathway and subsequently releasing a plethora of beneficial cytokines such as TGFβ. Figure 2 shows a schematic representation of astrocyte subtypes in AD.

2.9 Astrocytic Biomarkers in AD Patients

A biomarker may be defined as a biological molecule whose quantitative presence defines the sign of a normal biological process or a pathologic process or a disease. Sensitive AD-specific biomarkers are available that detect Aβ and tau proteins which are the main pathological features of the disease. By quantifying the CSF levels of soluble Aβ and tau or by imaging fibrillar form of Aβ and tau with positron emission tomography (PET) and detecting hypometabolism by [18F]flurodeoxyglucoe-PET or assessing the atrophy of the brain by structural magnetic resonance imaging (MRI), the cases are generally classified as positive or negative. However, none of these investigations are specific to astrocytes and are mostly used in the advanced stages of the disease. Astrocyte reactivity is an early pathological feature of AD; thus imaging or CSF biomarkers of reactive astrocyte would be critical in early diagnosis of AD and in monitoring the disease progression.

For AD, fluid biomarkers are usually quantified from the blood (plasma/serum) or CSF. Detection of biomarkers in blood is generally preferred as it is a low invasive process with the least side effects and can be easily obtained from the patients. A number of studies have revealed that there was a positive correlation between S100β and GFAP in blood with the late-onset sporadic AD patients, which was however lacking in the early onset AD patients. Furthermore, reduced levels of S100β was detected in the blood of late-onset AD patients in comparison to controls, although it was significantly increased with worsening dementia severity within the AD group (Lam et al. 2013; Mecocci et al. 1995; Chaves et al. 2010). CSF collection from patients involving lumbar puncture is a more invasive process than collection of blood. With regard to GFAP and S100β in CSF, GFAP levels were found to be higher in AD and other dementias, whereas, S100β levels were found to be lower in AD compared to control (Perez-Nievas and Serrano-Pozo 2018). However, some proteomic approaches have been shown to be promising for evaluating CSF biomarkers of astrocyte origin. For example, levels of an astrocyte-secreted gliotransmitter, D-serine, were elevated in probable AD patients (Madeira et al. 2015). Another well recognized CSF biomarker of astrocyte origin is YKL-40 protein overexpressed in a particular astrocytic subset in AD patients as opposed to the control patients (Querol-Vilaseca et al. 2017). It has been shown to predict progression from preclinical stage to AD dementia. Moreover, a positive correlation has been found between CSF YKL-40 levels and p-tau and t-tau levels at the early stages of AD (Alcolea et al. 2014, 2015; Craig-Schapiro et al. 2010; Antonell et al. 2014).

Another very important tool to measure biomarkers in vivo is imaging using PET and MRI techniques. It has been possible to check neuroinflammation by PET scan of the 18-kDa translocator protein (TSPO) which has been found to be increased within outer mitochondrial membrane in activated microglia. Interestingly, TSPO is expressed in reactive astrocytes in AD animals and postmortem brain of AD patients; however, it is yet to be recognized as an astrocyte biomarker (Cosenza-Nashat et al. 2009; Lavisse et al. 2012). Currently, two PET radiotracers, namely, [11C]-deuterium-L-deprenyl ([11C]DED) (Fowler et al. 1995) and [11C]BU99008 (Tyacke

et al. 2012), have been proposed to check biomarkers of reactive astrocytes. The radiotracer ([11C]DED, a modified inhibitor of the MAO-B enzyme, was used in several neurological diseases including AD (Carter et al. 2019). In transgenic mouse models, Aβ deposition is associated with an upregulation of MAO-B enzyme at early stages of AD unlike the advanced stages of the disease where it is not elevated as compared to other biomarkers such as GFAP (Olsen et al. 2018; Rodriguez-Vieitez et al. 2015). Human data showed that [11C]DED binding to MAO-B is enhanced in the early stages in AD in comparison to controls (Carter et al. 2012). The PET radiotracer [11C]BU99008 measures imidazoline 2-binding sites that are present on mitochondrial membranes within astrocytes and are found to be elevated in post-mortem AD brains (Parker et al. 2014).

Development of an in vivo astrocyte biomarker for MRI is highly recommended because of its widespread availability and convenience. Myoinositol which is known to be widely present in astrocyte and also in astrocyte-to-neuron shuttling process is determined by calculating the lactate and glutamate levels in rodents. Measuring myoinositol in vivo with [1H]-magnetic resonance spectroscopy (MRS) would be useful to assess the overall astrocyte integrity and structure (Harris et al. 2015). In a study antemortem MRS was compared to postmortem immunohistochemistry, and elevated myoinositol level was detected (scaled by creatine; myo-inositol/creatinine) in the cingulate cortex close to the areas of Aβ deposition in AD (Murray et al. 2014).

It is now well accepted that astrocyte biomarkers which are highly sensitive and specific to AD and can be assessed easily with neuroimaging or detected in body fluids will be valuable in complementing the commonly used AD biomarkers. Developing novel in vivo astrocyte biomarkers will help in quantifying astrocytes reactivity at early stages of AD and would help to understand the disease pathogenesis more precisely.

2.10 Astrocytes as Targets for Therapy in AD

From the foregone, it is established that it is the inability of astrocytes to protect the neurons against pathological insults caused by Aβ accumulation or a shift from physiological homeostatic support of CNS neurons by astrocytes under a stressed environment is the major reason for AD evolution and subsequent cognitive deficits. As discussed earlier, astrogliosis is an evidently controversial phenomenon, and the disparity between the roles played by the reactive astrocytes must be questioned in order to design an astro-centric therapy in AD. It is thus imperative to ask—when do the reactive astrocytes lose their initial defensive property during AD progression? What leads to the exhaustion of its defensive capabilities vividly evident in the early stages of AD? Most importantly, what is the definitive switch that alters the reactive astrocytes from being protective to detrimental during the progression from an early stage to the later stages in AD? Thus, cell-specific therapies that involve boosting the neuroprotective/defensive capabilities of the reactive astrocytes in AD and/or inhibiting the astroglial gain-of-toxic functions or loss of protective abilities may

provide new opportunities for prevention, arrest, or cure of AD (Verkhratsky et al. 2019b).

Astrocyte Subtype-Based Therapy

Utilizing the knowledge that astrocytes have different reactive subtypes, new methods in astro-centric therapy in AD may be developed. Astrocyte subtype-based approach in AD therapy is gaining momentum (Arranz and De Strooper 2019). Especially blocking the formation of the various types of detrimental reactive astrocytes may be an interesting approach in AD and other related disease therapy (Arranz and De Strooper 2019; Liddelow et al. 2017). Further, promoting the neuroprotective astrocyte phenotype may be of particular interest since these reactive astrocytes are involved in an upregulated uptake and clearance of Aβ (Liddelow et al. 2017). So utilizing drugs which could influence the Aβ clearance by astrocytes via ubiquitination or autophagy could have a therapeutic possibility in AD treatment. Moreover, beneficial astrocytes are known to induce Nrf2 transcription factor, a trophic factor which helps in activating antioxidant systems crucial for proper brain functioning, and could be targeted in AD therapy. Of note, as already discussed, following Aβ uptake if the reactive astrocytes are unable to degrade it, they may start producing upregulated levels of reactive oxygen species (ROS), inhibitory gliotransmitters, as well as other toxic products including an overproduction of Aβ itself, thus transforming to its detrimental phenotype. Hence, drugs regulating ROS production or triggering the antioxidant pathways in astrocytes could also be utilized for AD treatment. Moreover, regulating MAO-B activity, which controls the astrocytic GABA levels, could also ameliorate the memory deficits in AD (Chun and Lee 2018).

Following the A1-like astrocyte inhibition approach, a recent report suggests that microglial activation, which subsequently leads to the transformation to toxic A1-like astrocytes, can be prevented by applying the long-acting glucagon-like peptide-1 receptor (GLP1R) agonist known as the NLY01 (Yun et al. 2018). Thus, GLP1R agonists have evolved as potential neuroprotective molecules in AD and other types of neurodegenerative diseases' treatment. Moreover, inhibiting C3 or blocking C3aR, its receptor, has also been considered as a therapeutic approach (Lian et al. 2016) for inhibiting A1-like astrocytes. Even though most of the experiments have only been done in cell culture models, recent studies have shown that the deletion of C3aR genetically or using antagonist against C3aR has restored cognitive functioning in transgenic APPswe mice (Lian and Zheng 2016). It has been noted that the reactive astroglia especially those near the sites of Aβ plaques show an abundance of purinergic receptor P2Y1. One of the studies show that (Reichenbach et al. 2018) antagonists of these P2Y1 may inhibit the astrocyte hyperactivity in an AD mouse model. The major benefits for this treatment include the restoration of neuronal-astroglial network functioning and normalization of synaptic integrity and reduction in neuritic dystrophy which finally improves the cognitive decline in the rodents. Thus, targeting subtype-specific astrocytic mechanisms may be a significant approach in AD therapy. All the potential

astrocyte-subtype-specific therapies in AD are discussed by us in a recently published review (Sarkar and Biswas 2021).

Neuroinflammatory Cytokines as Therapeutic Targets

The other approach may be to target the specific neuroinflammatory cytokines secreted by the reactive microglia—IL-1α, TNFα, and C1q—that eventually lead to development of A1-like phenotype (Arranz and De Strooper 2019). TNFα inhibition may be thus a possible mode of AD therapy as its antagonism can prevent astroglial conversion to the A1-like phenotype. A reduction in the relative risk of AD development was found with the use of etanercept, a TNFα inhibitor, in rheumatoid arthritis patients, as compared to healthy untreated controls (Chou et al. 2016; Ekert et al. 2018). However, an inconclusive phase 2 trial in AD patients (Decourt et al. 2017) and extraordinary yet isolated case studies were not enough to validate Etanercept as an AD drug (Ekert et al. 2018). Similarly, a recombinant antagonist against IL-1α (anakinra) and an anti-C1q antibody are in line for clinical trials in AD treatment (Liddelow and Barres 2017; Lansita et al. 2017). Activated astrocytes are the major secretory cells which induce the production of various pro-inflammatory cytokines like IL-1β, IL-6, and IFN-γ which influence the surrounding neurons towards degeneration by upregulating the caspase-3 activity and cleavage of hyperphosphorylated tau protein. Recently a drug named minocycline which targets the increased production of cytokines was tried and a significant reduction in neuronal degeneration was observed even in presence of Aβ (Garwood et al. 2010). So it may well be said that cytokine releasing property of astrocytes can be targeted to treat AD patients.

Given the role of astrocytes in neuroinflammation in AD, exceptional reduction in glial activation and thus in AD progression has been seen in animal models by application of nonsteroidal anti-inflammatory drugs (NSAIDs) (Sastre and Gentleman 2010; Gasparini et al. 2004). Reports also suggest that agonists of PPAR-γ, the target receptor of NSAIDs that helps attenuate glial cell activation and the associated release of pro-inflammatory cytokines, namely, pioglitazone or GFT1803 displays positive effects in restricting the neuronal degeneration in AD, through reduction in Aβ plaque deposition and astrocytic reactivation (Heneka et al. 2005; de Jong et al. 2008). However, beyond the preclinical success, clinical trials with these drugs for AD treatment mostly proved to be a failure, again highlighting that the treatment with these anti-inflammatory drugs should be AD stage-dependent. Additionally, in vitro studies revealed the negative impact of glucocorticoids, with known anti-inflammatory properties, on cortical astrocyte proliferation (Crossin et al. 1997). The glucocorticoids also attenuated the release of pro-inflammatory cytokines that we know now are mostly released by the reactive A1-like astrocytes, in mice upon treatment with Aβ or LPS. However, glucocorticoids again failed to show any definite success in clinical trials (Fakhoury 2018). Sinomenine, a compound isolated from a Chinese medicinal plant, *Sinomenium acutum*, was shown to block the generation of nitric oxide (NO), ROS, and pro-inflammatory molecules from reactive astrocytes (probably, A1-like astrocytes) and protected hippocampal neurons against astrocyte-mediated toxicity (Singh et al. 2020). However, further validations

of this compound in clinical trials are warranted. Contrastingly, Heneka et al. (Heneka et al. 2013) observed that upon inhibition of NLRP3 inflammasome in APP/PS1 mice, the microglial M2 phenotype was bolstered giving evidence in support of similar A2-like astrocyte targeted therapy for AD. Interestingly, the transplantation of lineage negative stem cells in an intrahippocampally Aβ-infused mouse resulted in recovery of impaired memory with reduction of Aβ accumulation. It is probably mediated by the A2-like astrocytes with the influence of neurotrophic factors such as glial derived neurotrophic factor (GDNF), ciliary derived neurotrophic factor (CNTF), and brain-derived neurotrophic factor (BDNF) (Bali et al. 2019).

Targeting Metabolic Dysfunctions for Therapy

Glutamate excitotoxicity results from a reduced glutamate clearance by astrocytes from the synaptic cleft in AD. This problem was attributed to the reduced or mislocated GLT1 expression on the reactive astrocytes. Thus, drugs that can induce an enhanced expression of astrocytic glutamate transporters may be a potential mode of AD treatment targeting excitotoxicity (Assefa et al. 2018). β-Lactam antibiotics including penicillin and its other derivatives particularly stimulate GLT1 expression (Rothstein et al. 2005). Ceftriaxone-mediated elevated expression of astrocytic GLT1 was found to ameliorate learning and memory in rat model of AD. Other compounds like estrogen, ampicillin, insulin, and riluzole were also observed to induce GLT1 expression. GLT1 expression inducers may also influence AD progression alone or along with already marketed NMDA antagonists, but this strategy needs to be investigated further. Ceftriaxone is slated for clinical trial in treatment of AD (Assefa et al. 2018). It has been previously discussed that reactive astrocytes can induce excessive tonic inhibition by enhanced GABA production and thus drugs reducing this induction may present yet another way in restricting AD progression. Thus it is important to develop drugs that can either block GABA receptor or block the synthesis and release of GABA from the reactive astrocytes (Assefa et al. 2018). L-655708, a GABA receptor inverse agonist, was able to improve the cognitive deficits in mice models of AD (Wu et al. 2014). Another pathway that may be targeted is the calcineurin/NFAT pathway since its inhibition in reactive astrocytes has shown attenuation in astrogliosis and subsequent amelioration of cognitive functions. Furman et al. (2012) showed that AAV vectors targeting hippocampal astrocytes in an AD mice model and inducing expression of VIVIT, a peptide interfering with Calcineurin/NFAT signaling pathway, further caused a reduction in astrocytic activation. Following a several-month-long treatment with VIVIT, the transgenic AD mice started displaying diminished Aβ load, reduced glial activation and significant restoration of cognitive functions (Furman et al. 2012).

Aquaporin as a Therapeutic Target

A well-known player in astrocytes relevant to neurological disorders is AQ4, which helps in various astrocytic functions, including glutamate homeostasis, astrocyte-mediated glial scarring, cellular communication, potassium uptake, synapse formation, etc. (Lan et al. 2016; Papadopoulos and Verkman 2013), is also known to

contribute to the clearance of Aβ by regulating the passage of various interstitial fluids which help in Aβ clearance (Iliff et al. 2012; Asgari et al. 2015). However, few reports indicate a possible detrimental role of AQ4 in accentuating the proinflammatory phenotype of astrocytes in AD (Li et al. 2011b; Huang et al. 2011). Even though no drug has been designed mimicking the beneficial aspect of the protein, yet, there is an immense scope in exploring AQ4 for the development of novel therapeutic strategies.

Hence, there are various strategies for restoring astrocytic functions following an astro-centric therapy in AD. However, identifying the specific stage during AD progression that would be ideal for targeting astrocytes for AD treatment is crucial.

3 Conclusions

AD research has had a primarily neuron-centric approach. In the beginning of this century, astrocytes have emerged as a crucial candidate in AD pathophysiology. Astrocytes, the most abundant cell type in CNS not only execute the normal homeostatic functioning in brain with aplomb but may also turn as a savior in the face of toxic insults such as Aβ accumulation. However, majority of the reports also emphasize the propelling role of astrocytes in AD progression. The recent discovery of heterogeneity of reactive states of astrocytes upholds the need for better and nuanced understanding of reactive astrogliosis with all its complexities in AD evolution. Indeed, conventionally generalized astrocytic response such as "astrogliosis" or "astrocytic reactivity" is being increasingly recognized as a rather complex and multifaceted phenomenon in various neurological diseases including AD comprising both neurotrophic and neurotoxic responses. Several aspects of reactive astrocytes in AD progression remain controversial till date and attract urgent attention towards critical delineation of each of the reactive states and better understanding of their altering phenotype and secretion profiles in response to pathological insults like Aβ. This chapter aims to disentangle this complex response from various aspects of AD including neuroinflammation, metabolic dysregulation, synaptic plasticity, neurotransmitter recycling, and tau pathology.

Astrocytes seem to play a key role in recently proposed cellular phase of "amyloid cascade hypothesis" of AD. The reactive astrocytes may either induce Aβ uptake or its clearance depending on the disease state. Evidence of neuroinflammation and metabolic dysregulation as a trigger for AD prior to Aβ deposition have been discussed. We also discussed the major signaling pathways of reactivated astrocytes playing critical role in the events that follow including Aβ generation/clearance, tau pathology, neurotransmitter recycling, synaptic health, and neuronal death/survival and cognitive functions. Intriguingly, through this chapter, it was concurrently established from all the abovementioned perspectives that reactive astrocytes present a defensive front during the initial stage of AD but turn detrimental at the later stages in disease progression. Hence, concentrated efforts must be made to discover the switch that modulates this transition, as well as to specifically determine the time point in AD progression when this transformation of astrocyte

responses occurs. Finally, the recent efforts in developing astrocyte-based biomarkers for AD and therapeutic strategies have been discussed. Reactive astrocyte-subtype-based therapies clearly emerge at the forefront of the cell-specific therapies aimed at instigating the defensive capabilities of the protective astrocyte subtype while paralyzing the detrimental subtypes. In fact, the association of the neuroprotective subtype with the early stage of AD presents an opportunity to arrest or even prevent the disease in its bud. Thus, this chapter emphasizes the significance of reactive astrocytes in AD progression, its diagnosis or even in its treatment. Last but not the least, astrocyte-subtype-specific transcriptomics or proteomics can divulge a huge array of therapeutic targets for AD in the near future.

Acknowledgements The authors would like to thank Sammani Sarkar for Artwork. The work was supported by CSIR, Govt. of India, and by the Department of Science and Technology, Govt. of India.

References

Abe K, Misawa M (2003) Amyloid beta protein enhances the clearance of extracellular L-glutamate by cultured rat cortical astrocytes. Neurosci Res 45(1):25–31

Abeti R, Abramov AY, Duchen MR (2011) Beta-amyloid activates PARP causing astrocytic metabolic failure and neuronal death. Brain 134(Pt 6):1658–1672

Acosta C, Anderson HD, Anderson CM (2017) Astrocyte dysfunction in Alzheimer disease. J Neurosci Res 95(12):2430–2447

Akama KT, Albanese C, Pestell RG, Van Eldik LJ (1998) Amyloid beta-peptide stimulates nitric oxide production in astrocytes through an NFkappaB-dependent mechanism. Proc Natl Acad Sci U S A 95(10):5795–5800

Alcolea D, Carmona-Iragui M, Suarez-Calvet M, Sanchez-Saudinos MB, Sala I, Anton-Aguirre S et al (2014) Relationship between beta-secretase, inflammation and core cerebrospinal fluid biomarkers for Alzheimer's disease. J Alzheimers Dis 42(1):157–167

Alcolea D, Martinez-Lage P, Sanchez-Juan P, Olazaran J, Antunez C, Izagirre A et al (2015) Amyloid precursor protein metabolism and inflammation markers in preclinical Alzheimer disease. Neurology 85(7):626–633

Alkam T, Nitta A, Mizoguchi H, Saito K, Seshima M, Itoh A, Yamada K, Nabeshima T (2008) Restraining tumor necrosis factor-alpha by thalidomide prevents the amyloid beta-induced impairment of recognition memory in mice. Behav Brain Res 189(1):100–106

Allaman I, Gavillet M, Belanger M, Laroche T, Viertl D, Lashuel HA, Magistretti PJ (2010) Amyloid-beta aggregates cause alterations of astrocytic metabolic phenotype: impact on neuronal viability. J Neurosci 30(9):3326–3338

Anderson CM, Bridges RJ, Chamberlin AR, Shimamoto K, Yasuda-Kamatani Y, Swanson RA (2001) Differing effects of substrate and non-substrate transport inhibitors on glutamate uptake reversal. J Neurochem 79(6):1207–1216

Anderson MA, Ao Y, Sofroniew MV (2014) Heterogeneity of reactive astrocytes. Neurosci Lett 565:23–29

Andriezen WL (1893) The neuroglia elements in the human brain. Br Med J 2(1700):227–230

Antonell A, Mansilla A, Rami L, Llado A, Iranzo A, Olives J, Balasa M, Sanchez-Valle R, Molinuevo JL (2014) Cerebrospinal fluid level of YKL-40 protein in preclinical and prodromal Alzheimer's disease. J Alzheimers Dis 42(3):901–908

Apelt J, Ach K, Schliebs R (2003) Aging-related down-regulation of neprilysin, a putative beta-amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied

by an astroglial upregulation in the vicinity of beta-amyloid plaques. Neurosci Lett 339(3): 183–186
Arranz AM, De Strooper B (2019) The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. Lancet Neurol 18(4):406–414
Asgari N, Berg CT, Morch MT, Khorooshi R, Owens T (2015) Cerebrospinal fluid aquaporin-4-immunoglobulin G disrupts blood brain barrier. Ann Clin Transl Neurol 2(8):857–863
Assefa BT, Gebre AK, Altaye BM (2018) Reactive astrocytes as drug target in Alzheimer's disease. Biomed Res Int 2018:4160247
Bales KR, Du Y, Dodel RC, Yan GM, Hamilton-Byrd E, Paul SM (1998) The NF-kappaB/Rel family of proteins mediates Abeta-induced neurotoxicity and glial activation. Brain Res Mol Brain Res 57(1):63–72
Bali P, Banik A, Nehru B, Anand A (2019) Neurotrophic factors mediated activation of astrocytes ameliorate memory loss by amyloid clearance after transplantation of lineage negative stem cells. Mol Neurobiol 56(12):8420–8434
Ballatore C, Lee VM, Trojanowski JQ (2007) Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci 8(9):663–672
Basak JM, Verghese PB, Yoon H, Kim J, Holtzman DM (2012) Low-density lipoprotein receptor represents an apolipoprotein E-independent pathway of Abeta uptake and degradation by astrocytes. J Biol Chem 287(17):13959–13971
Battaglia F, Wang HY, Ghilardi MF, Gashi E, Quartarone A, Friedman E, Nixon RA (2007) Cortical plasticity in Alzheimer's disease in humans and rodents. Biol Psychiatry 62(12): 1405–1412
Belanger M, Magistretti PJ (2009) The role of astroglia in neuroprotection. Dialogues Clin Neurosci 11(3):281–295
Benzing WC, Wujek JR, Ward EK, Shaffer D, Ashe KH, Younkin SG, Brunden KR (1999) Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. Neurobiol Aging 20(6):581–589
Bero AW, Yan P, Roh JH, Cirrito JR, Stewart FR, Raichle ME, Lee JM, Holtzman DM (2011) Neuronal activity regulates the regional vulnerability to amyloid-beta deposition. Nat Neurosci 14(6):750–756
Bigl M, Bruckner MK, Arendt T, Bigl V, Eschrich K (1999) Activities of key glycolytic enzymes in the brains of patients with Alzheimer's disease. J Neural Transm (Vienna) 106(5–6):499–511
Blasko I, Veerhuis R, Stampfer-Kountchev M, Saurwein-Teissl M, Eikelenboom P, Grubeck-Loebenstein B (2000) Costimulatory effects of interferon-gamma and interleukin-1beta or tumor necrosis factor alpha on the synthesis of Abeta1-40 and Abeta1-42 by human astrocytes. Neurobiol Dis 7:682–689
Blonz ER (2017) Alzheimer's disease as the product of a progressive energy deficiency syndrome in the central nervous system: the neuroenergetic hypothesis. J Alzheimers Dis 60(4): 1223–1229
Brawek B, Chesters R, Klement D, Muller J, Lerdkrai C, Hermes M, Garaschuk O (2018) A bell-shaped dependence between amyloidosis and GABA accumulation in astrocytes in a mouse model of Alzheimer's disease. Neurobiol Aging 61:187–197
Cai Z, Wan CQ, Liu Z (2017) Astrocyte and Alzheimer's disease. J Neurol 264(10):2068–2074
Carbone I, Lazzarotto T, Ianni M, Porcellini E, Forti P, Masliah E, Gabrielli L, Licastro F (2014) Herpes virus in Alzheimer's disease: relation to progression of the disease. Neurobiol Aging 35(1):122–129
Carter SF, Herholz K, Rosa-Neto P, Pellerin L, Nordberg A, Zimmer ER (2019) Astrocyte biomarkers in Alzheimer's disease. Trends Mol Med 25(2):77–95
Carter SF, Scholl M, Almkvist O, Wall A, Engler H, Langstrom B, Nordberg A (2012) Evidence for astrocytosis in prodromal Alzheimer disease provided by 11C-deuterium-L-deprenyl: a multitracer PET paradigm combining 11C-Pittsburgh compound B and 18F-FDG. J Nucl Med 53(1):37–46

Ceyzeriat K, Ben Haim L, Denizot A, Pommier D, Matos M, Guillemaud O et al (2018) Modulation of astrocyte reactivity improves functional deficits in mouse models of Alzheimer's disease. Acta Neuropathol Commun 6(1):104

Chakrabarty P, Li A, Ceballos-Diaz C, Eddy JA, Funk CC, Moore B et al (2015) IL-10 alters immunoproteostasis in APP mice, increasing plaque burden and worsening cognitive behavior. Neuron 85(3):519–533

Chakraborty A, de Wit NM, van der Flier WM, de Vries HE (2017) The blood brain barrier in Alzheimer's disease. Vascul Pharmacol 89:12–18

Chaves ML, Camozzato AL, Ferreira ED, Piazenski I, Kochhann R, Dall'Igna O, Mazzini GS, Souza DO, Portela LV (2010) Serum levels of S100B and NSE proteins in Alzheimer's disease patients. J Neuroinflammation 7:6

Cheng L, Yin WJ, Zhang JF, Qi JS (2009) Amyloid beta-protein fragments 25-35 and 31-35 potentiate long-term depression in hippocampal CA1 region of rats in vivo. Synapse 63(3): 206–214

Chou RC, Kane M, Ghimire S, Gautam S, Gui J (2016) Treatment for rheumatoid arthritis and risk of Alzheimer's disease: a nested case-control analysis. CNS Drugs 30(11):1111–1120

Chun H, Lee CJ (2018) Reactive astrocytes in Alzheimer's disease: a double-edged sword. Neurosci Res 126:44–52

Cosenza-Nashat M, Zhao ML, Suh HS, Morgan J, Natividad R, Morgello S, Lee SC (2009) Expression of the translocator protein of 18 kDa by microglia, macrophages and astrocytes based on immunohistochemical localization in abnormal human brain. Neuropathol Appl Neurobiol 35(3):306–328

Cotto B, Natarajaseenivasan K, Langford D (2019) Astrocyte activation and altered metabolism in normal aging, age-related CNS diseases, and HAND. J Neurovirol 25(5):722–733

Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, Cairns NJ et al (2010) YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. Biol Psychiatry 68(10):903–912

Crossin KL, Tai MH, Krushel LA, Mauro VP, Edelman GM (1997) Glucocorticoid receptor pathways are involved in the inhibition of astrocyte proliferation. Proc Natl Acad Sci U S A 94(6):2687–2692

Das S, Li Z, Noori A, Hyman BT, Serrano-Pozo A (2020) Meta-analysis of mouse transcriptomic studies supports a context-dependent astrocyte reaction in acute CNS injury versus neurodegeneration. J Neuroinflammation 17(1):227

de Jong D, Jansen R, Hoefnagels W, Jellesma-Eggenkamp M, Verbeek M, Borm G, Kremer B (2008) No effect of one-year treatment with indomethacin on Alzheimer's disease progression: a randomized controlled trial. PLoS One 3(1):e1475

De Santi S, de Leon MJ, Rusinek H, Convit A, Tarshish CY, Roche A et al (2001) Hippocampal formation glucose metabolism and volume losses in MCI and AD. Neurobiol Aging 22(4): 529–539

De Strooper B, Karran E (2016) The cellular phase of Alzheimer's disease. Cell 164(4):603–615

de Vivo L, Melone M, Rothstein JD, Conti F (2010) GLT-1 promoter activity in astrocytes and neurons of mouse hippocampus and somatic sensory cortex. Front Neuroanat 3:31

Decourt B, Lahiri DK, Sabbagh MN (2017) Targeting tumor necrosis factor alpha for Alzheimer's disease. Curr Alzheimer Res 14(4):412–425

Duran J, Saez I, Gruart A, Guinovart JJ, Delgado-Garcia JM (2013) Impairment in long-term memory formation and learning-dependent synaptic plasticity in mice lacking glycogen synthase in the brain. J Cereb Blood Flow Metab 33(4):550–556

Eid T, Behar K, Dhaher R, Bumanglag AV, Lee TS (2012) Roles of glutamine synthetase inhibition in epilepsy. Neurochem Res 37(11):2339–2350

Ekert JO, Gould RL, Reynolds G, Howard RJ (2018) TNF alpha inhibitors in Alzheimer's disease: a systematic review. Int J Geriatr Psychiatry 33(5):688–694

Escartin C, Galea E, Lakatos A, O'Callaghan JP, Petzold GC, Serrano-Pozo A et al (2021) Reactive astrocyte nomenclature, definitions, and future directions. Nat Neurosci 24(3):312–325

Escartin C, Guillemaud O, Carrillo-de Sauvage MA (2019) Questions and (some) answers on reactive astrocytes. Glia 67(12):2221–2247

Escartin C, Pierre K, Colin A, Brouillet E, Delzescaux T, Guillermier M et al (2007) Activation of astrocytes by CNTF induces metabolic plasticity and increases resistance to metabolic insults. J Neurosci 27(27):7094–7104

Fakhoury M (2018) Microglia and astrocytes in Alzheimer's disease: implications for therapy. Curr Neuropharmacol 16(5):508–518

Falkowska A, Gutowska I, Goschorska M, Nowacki P, Chlubek D, Baranowska-Bosiacka I (2015) Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. Int J Mol Sci 16(11):25959–25981

Fernandez-Tome P, Brera B, Arevalo MA, de Ceballos ML (2004) Beta-amyloid25-35 inhibits glutamate uptake in cultured neurons and astrocytes: modulation of uptake as a survival mechanism. Neurobiol Dis 15(3):580–589

Forster S, Grimmer T, Miederer I, Henriksen G, Yousefi BH, Graner P et al (2012) Regional expansion of hypometabolism in Alzheimer's disease follows amyloid deposition with temporal delay. Biol Psychiatry 71(9):792–797

Fowler JS, Wang GJ, Logan J, Xie S, Volkow ND, MacGregor RR et al (1995) Selective reduction of radiotracer trapping by deuterium substitution: comparison of carbon-11-L-deprenyl and carbon-11-deprenyl-D2 for MAO B mapping. J Nucl Med 36(7):1255–1262

Frost GR, Li YM (2017) The role of astrocytes in amyloid production and Alzheimer's disease. Open Biol 7:12

Fu W, Shi D, Westaway D, Jhamandas JH (2015) Bioenergetic mechanisms in astrocytes may contribute to amyloid plaque deposition and toxicity. J Biol Chem 290(20):12504–12513

Funato H, Yoshimura M, Yamazaki T, Saido TC, Ito Y, Yokofujita J, Okeda R, Ihara Y (1998) Astrocytes containing amyloid beta-protein (Abeta)-positive granules are associated with Abeta40-positive diffuse plaques in the aged human brain. Am J Pathol 152(4):983–992

Furman JL, Sama DM, Gant JC, Beckett TL, Murphy MP, Bachstetter AD, Van Eldik LJ, Norris CM (2012) Targeting astrocytes ameliorates neurologic changes in a mouse model of Alzheimer's disease. J Neurosci 32(46):16129–16140

Garcia-Esparcia P, Diaz-Lucena D, Ainciburu M, Torrejon-Escribano B, Carmona M, Llorens F, Ferrer I (2018) Glutamate transporter GLT1 expression in Alzheimer disease and dementia with Lewy bodies. Front Aging Neurosci 10:122

Garwood CJ, Cooper JD, Hanger DP, Noble W (2010) Anti-inflammatory impact of minocycline in a mouse model of tauopathy. Front Psychiatry 1:136

Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W (2011) Astrocytes are important mediators of Abeta-induced neurotoxicity and tau phosphorylation in primary culture. Cell Death Dis 2:e167

Garwood CJ, Ratcliffe LE, Simpson JE, Heath PR, Ince PG, Wharton SB (2017) Review: astrocytes in Alzheimer's disease and other age-associated dementias: a supporting player with a central role. Neuropathol Appl Neurobiol 43(4):281–298

Gasparini L, Ongini E, Wenk G (2004) Non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease: old and new mechanisms of action. J Neurochem 91(3):521–536

Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N (2010) Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 11(2):87–99

Gibson EM, Nagaraja S, Ocampo A, Tam LT, Wood LS, Pallegar PN et al (2019) Methotrexate chemotherapy induces persistent tri-glial dysregulation that underlies chemotherapy-related cognitive impairment. Cell 176:e13

Gomez-Arboledas A, Davila JC, Sanchez-Mejias E, Navarro V, Nunez-Diaz C, Sanchez-Varo R et al (2018) Phagocytic clearance of presynaptic dystrophies by reactive astrocytes in Alzheimer's disease. Glia 66(3):637–653

Gong CX, Liu F, Grundke-Iqbal I, Iqbal K (2006) Impaired brain glucose metabolism leads to Alzheimer neurofibrillary degeneration through a decrease in tau O-GlcNAcylation. J Alzheimers Dis 9(1):1–12

Gonzalez-Reyes RE, Nava-Mesa MO, Vargas-Sanchez K, Ariza-Salamanca D, Mora-Munoz L (2017) Involvement of astrocytes in Alzheimer's disease from a Neuroinflammatory and oxidative stress perspective. Front Mol Neurosci 10:427

Grolla AA, Fakhfouri G, Balzaretti G, Marcello E, Gardoni F, Canonico PL, DiLuca M, Genazzani AA, Lim D (2013a) Abeta leads to ca(2)(+) signaling alterations and transcriptional changes in glial cells. Neurobiol Aging 34(2):511–522

Grolla AA, Sim JA, Lim D, Rodriguez JJ, Genazzani AA, Verkhratsky A (2013b) Amyloid-beta and Alzheimer's disease type pathology differentially affects the calcium signalling toolkit in astrocytes from different brain regions. Cell Death Dis 4:e623

Grubman A, Chew G, Ouyang JF, Sun G, Choo XY, McLean C et al (2019) A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. Nat Neurosci 22(12):2087–2097

Guillot-Sestier MV, Doty KR, Gate D, Rodriguez J Jr, Leung BP, Rezai-Zadeh K, Town T (2015) Il10 deficiency rebalances innate immunity to mitigate Alzheimer-like pathology. Neuron 85(3): 534–548

Habbas S, Santello M, Becker D, Stubbe H, Zappia G, Liaudet N et al (2015) Neuroinflammatory TNFalpha impairs memory via astrocyte signaling. Cell 163(7):1730–1741

Habib N, McCabe C, Medina S, Varshavsky M, Kitsberg D, Dvir-Szternfeld R et al (2020) Disease-associated astrocytes in Alzheimer's disease and aging. Nat Neurosci 23(6):701–706

Harkany T, Abraham I, Timmerman W, Laskay G, Toth B, Sasvari M et al (2000) Beta-amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis. Eur J Neurosci 12(8):2735–2745

Harris JL, Choi IY, Brooks WM (2015) Probing astrocyte metabolism in vivo: proton magnetic resonance spectroscopy in the injured and aging brain. Front Aging Neurosci 7:202

Hartlage-Rubsamen M, Zeitschel U, Apelt J, Gartner U, Franke H, Stahl T et al (2003) Astrocytic expression of the Alzheimer's disease beta-secretase (BACE1) is stimulus-dependent. Glia 41(2):169–179

Haughey NJ, Mattson MP (2003) Alzheimer's amyloid beta-peptide enhances ATP/gap junction-mediated calcium-wave propagation in astrocytes. Neuromolecular Med 3(3):173–180

Hefendehl JK, LeDue J, Ko RW, Mahler J, Murphy TH, MacVicar BA (2016) Mapping synaptic glutamate transporter dysfunction in vivo to regions surrounding Abeta plaques by iGluSnFR two-photon imaging. Nat Commun 7:13441

Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL et al (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol 14(4):388–405

Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A et al (2013) NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493(7434):674–678

Heneka MT, Sastre M, Dumitrescu-Ozimek L, Hanke A, Dewachter I, Kuiperi C et al (2005) Acute treatment with the PPARgamma agonist pioglitazone and ibuprofen reduces glial inflammation and Abeta1-42 levels in APPV717I transgenic mice. Brain 128(Pt 6):1442–1453

Hong P, Jiang M, Li H (2014) Functional requirement of dicer1 and miR-17-5p in reactive astrocyte proliferation after spinal cord injury in the mouse. Glia 62(12):2044–2060

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science 274(5284):99–102

Huang S, Tong H, Lei M, Zhou M, Guo W, Li G et al (2018) Astrocytic glutamatergic transporters are involved in Abeta-induced synaptic dysfunction. Brain Res 1678:129–137

Huang W, Xing W, Ryskamp DA, Punzo C, Krizaj D (2011) Localization and phenotype-specific expression of ryanodine calcium release channels in C57BL6 and DBA/2J mouse strains. Exp Eye Res 93(5):700–709

Husemann J, Silverstein SC (2001) Expression of scavenger receptor class B, type I, by astrocytes and vascular smooth muscle cells in normal adult mouse and human brain and in Alzheimer's disease brain. Am J Pathol 158(3):825–832

Hutchison ER, Kawamoto EM, Taub DD, Lal A, Abdelmohsen K, Zhang Y et al (2013) Evidence for miR-181 involvement in neuroinflammatory responses of astrocytes. Glia 61(7):1018–1028

Ikegaya Y, Matsuura S, Ueno S, Baba A, Yamada MK, Nishiyama N, Matsuki N (2002) Beta-amyloid enhances glial glutamate uptake activity and attenuates synaptic efficacy. J Biol Chem 277(35):32180–32186

Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA et al (2012) A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. Sci Transl Med 4:147

Iram T, Trudler D, Kain D, Kanner S, Galron R, Vassar R et al (2016) Astrocytes from old Alzheimer's disease mice are impaired in Abeta uptake and in neuroprotection. Neurobiol Dis 96:84–94

Ito M, Komai K, Mise-Omata S, Iizuka-Koga M, Noguchi Y, Kondo T et al (2019) Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. Nature 565(7738):246–250

Jacob CP, Koutsilieri E, Bartl J, Neuen-Jacob E, Arzberger T, Zander N et al (2007) Alterations in expression of glutamatergic transporters and receptors in sporadic Alzheimer's disease. J Alzheimers Dis 11(1):97–116

Jankowsky JL, Fadale DJ, Anderson J, Xu GM, Gonzales V, Jenkins NA et al (2004) Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. Hum Mol Genet 13(2):159–170

Ji HF, Shen L, Zhang HY (2005) Beta-lactam antibiotics are multipotent agents to combat neurological diseases. Biochem Biophys Res Commun 333(3):661–663

Jin SM, Cho HJ, Kim YW, Hwang JY, Mook-Jung I (2012) Abeta-induced ca(2+) influx regulates astrocytic BACE1 expression via calcineurin/NFAT4 signals. Biochem Biophys Res Commun 425(3):649–655

Jo S, Yarishkin O, Hwang YJ, Chun YE, Park M, Woo DH et al (2014) GABA from reactive astrocytes impairs memory in mouse models of Alzheimer's disease. Nat Med 20(8):886–896

Johnstone M, Gearing AJ, Miller KM (1999) A central role for astrocytes in the inflammatory response to beta-amyloid; chemokines, cytokines and reactive oxygen species are produced. J Neuroimmunol 93:182–193

Jones RS, Minogue AM, Connor TJ, Lynch MA (2013) Amyloid-beta-induced astrocytic phagocytosis is mediated by CD36, CD47 and RAGE. J Neuroimmune Pharmacol 8(1):301–311

Jung ES, An K, Hong HS, Kim JH, Mook-Jung I (2012) Astrocyte-originated ATP protects Abeta (1-42)-induced impairment of synaptic plasticity. J Neurosci 32(9):3081–3087

Kahlson MA, Colodner KJ (2015) Glial tau pathology in Tauopathies: functional consequences. J Exp Neurosci 9(Suppl 2):43–50

Katsouri L, Georgopoulos S (2011) Lack of LDL receptor enhances amyloid deposition and decreases glial response in an Alzheimer's disease mouse model. PLoS One 6(7):e21880

Kilian JG, Hsu HW, Mata K, Wolf FW, Kitazawa M (2017) Astrocyte transport of glutamate and neuronal activity reciprocally modulate tau pathology in drosophila. Neuroscience 348:191–200

Kim J, Castellano JM, Jiang H, Basak JM, Parsadanian M, Pham V, Mason SM, Paul SM, Holtzman DM (2009) Overexpression of low-density lipoprotein receptor in the brain markedly inhibits amyloid deposition and increases extracellular a beta clearance. Neuron 64(5):632–644

Kim YS, Jung HM, Yoon BE (2018) Exploring glia to better understand Alzheimer's disease. Anim Cells Syst 22(4):213–218

Kitazawa M, Cheng D, Tsukamoto MR, Koike MA, Wes PD, Vasilevko V, Cribbs DH, LaFerla FM (2011) Blocking IL-1 signaling rescues cognition, attenuates tau pathology, and restores neuronal beta-catenin pathway function in an Alzheimer's disease model. J Immunol 187(12): 6539–6549

Knobloch M, Konietzko U, Krebs DC, Nitsch RM (2007) Intracellular Abeta and cognitive deficits precede beta-amyloid deposition in transgenic arcAbeta mice. Neurobiol Aging 28(9): 1297–1306

Koenigsknecht-Talboo J, Landreth GE (2005) Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. J Neurosci 25(36): 8240–8249

Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J et al (2004) Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. Nat Med 10(7):719–726

Kraft AW, Hu X, Yoon H, Yan P, Xiao Q, Wang Y et al (2013) Attenuating astrocyte activation accelerates plaque pathogenesis in APP/PS1 mice. FASEB J 27(1):187–198

Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ (2009) Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. Science 323(5918):1211–1215

Kurt MA, Davies DC, Kidd M (1999) Beta-amyloid immunoreactivity in astrocytes in Alzheimer's disease brain biopsies: an electron microscope study. Exp Neurol 158(1):221–228

Lam AG, Koppal T, Akama KT, Guo L, Craft JM, Samy B, Schavocky JP, Watterson DM, Van Eldik LJ (2001) Mechanism of glial activation by S100B: involvement of the transcription factor NFkappaB. Neurobiol Aging 22(5):765–772

Lam V, Albrecht MA, Takechi R, Giles C, James AP, Foster JK, Mamo JC (2013) The serum concentration of the calcium binding protein S100B is positively associated with cognitive performance in older adults. Front Aging Neurosci 5:61

Lan YL, Zhao J, Ma T, Li S (2016) The potential roles of aquaporin 4 in Alzheimer's disease. Mol Neurobiol 53(8):5300–5309

Lansita JA, Mease KM, Qiu H, Yednock T, Sankaranarayanan S, Kramer S (2017) Nonclinical development of ANX005: a humanized anti-C1q antibody for treatment of autoimmune and neurodegenerative diseases. Int J Toxicol 36(6):449–462

Lavisse S, Guillermier M, Herard AS, Petit F, Delahaye M, Van Camp N et al (2012) Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. J Neurosci 32(32):10809–10818

Leal MC, Dorfman VB, Gamba AF, Frangione B, Wisniewski T, Castano EM, Sigurdsson EM, Morelli L (2006) Plaque-associated overexpression of insulin-degrading enzyme in the cerebral cortex of aged transgenic tg2576 mice with Alzheimer pathology. J Neuropathol Exp Neurol 65(10):976–987

Leyns CEG, Ulrich JD, Finn MB, Stewart FR, Koscal LJ, Remolina Serrano J et al (2017) TREM2 deficiency attenuates neuroinflammation and protects against neurodegeneration in a mouse model of tauopathy. Proc Natl Acad Sci U S A 114(43):11524–11529

Li C, Zhao R, Gao K, Wei Z, Yin MY, Lau LT, Chui D, Yu AC (2011a) Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. Curr Alzheimer Res 8(1):67–80

Li L, Zhang H, Varrin-Doyer M, Zamvil SS, Verkman AS (2011b) Proinflammatory role of aquaporin-4 in autoimmune neuroinflammation. FASEB J 25(5):1556–1566

Li S, Jin M, Koeglsperger T, Shepardson NE, Shankar GM, Selkoe DJ (2011c) Soluble Abeta oligomers inhibit long-term potentiation through a mechanism involving excessive activation of extrasynaptic NR2B-containing NMDA receptors. J Neurosci 31(18):6627–6638

Li S, Mallory M, Alford M, Tanaka S, Masliah E (1997) Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. J Neuropathol Exp Neurol 56(8):901–911

Lian H, Litvinchuk A, Chiang AC, Aithmitti N, Jankowsky JL, Zheng H (2016) Astrocyte-microglia cross talk through complement activation modulates amyloid pathology in mouse models of Alzheimer's disease. J Neurosci 36(2):577–589

Lian H, Zheng H (2016) Signaling pathways regulating neuron-glia interaction and their implications in Alzheimer's disease. J Neurochem 136(3):475–491

Liang WS, Reiman EM, Valla J, Dunckley T, Beach TG, Grover A et al (2008) Alzheimer's disease is associated with reduced expression of energy metabolism genes in posterior cingulate neurons. Proc Natl Acad Sci U S A 105(11):4441–4446

Liddelow SA, Barres BA (2017) Reactive astrocytes: production, function, and therapeutic potential. Immunity 46(6):957–967

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L et al (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541(7638):481–487
Lipton SA (2004) Failures and successes of NMDA receptor antagonists: molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. NeuroRx 1(1):101–110
Long JM, Holtzman DM (2019) Alzheimer disease: An update on pathobiology and treatment strategies. Cell 179(2):312–339
Lord A, Kalimo H, Eckman C, Zhang XQ, Lannfelt L, Nilsson LN (2006) The Arctic Alzheimer mutation facilitates early intraneuronal Abeta aggregation and senile plaque formation in transgenic mice. Neurobiol Aging 27(1):67–77
Madeira C, Lourenco MV, Vargas-Lopes C, Suemoto CK, Brandao CO, Reis T et al (2015) D-serine levels in Alzheimer's disease: implications for novel biomarker development. Transl Psychiatry 5:e561
Manev H, Favaron M, Guidotti A, Costa E (1989) Delayed increase of Ca2+ influx elicited by glutamate: role in neuronal death. Mol Pharmacol 36(1):106–112
Masliah E, Alford M, DeTeresa R, Mallory M, Hansen L (1996) Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. Ann Neurol 40(5):759–766
Matias I, Morgado J, Gomes FCA (2019) Astrocyte heterogeneity: impact to brain aging and disease. Front Aging Neurosci 11:59
Matos M, Augusto E, Machado NJ, dos Santos-Rodrigues A, Cunha RA, Agostinho P (2012) Astrocytic adenosine A2A receptors control the amyloid-beta peptide-induced decrease of glutamate uptake. J Alzheimers Dis 31(3):555–567
Matos M, Augusto E, Oliveira CR, Agostinho P (2008) Amyloid-beta peptide decreases glutamate uptake in cultured astrocytes: involvement of oxidative stress and mitogen-activated protein kinase cascades. Neuroscience 156(4):898–910
McGeer EG, McGeer PL (2010) Neuroinflammation in Alzheimer's disease and mild cognitive impairment: a field in its infancy. J Alzheimers Dis 19(1):355–361
Mecocci P, Parnetti L, Romano G, Scarelli A, Chionne F, Cecchetti R et al (1995) Serum anti-GFAP and anti-S100 autoantibodies in brain aging, Alzheimer's disease and vascular dementia. J Neuroimmunol 57(1–2):165–170
Merlini M, Meyer EP, Ulmann-Schuler A, Nitsch RM (2011) Vascular beta-amyloid and early astrocyte alterations impair cerebrovascular function and cerebral metabolism in transgenic arcAbeta mice. Acta Neuropathol 122(3):293–311
Mizee MR, Nijland PG, van der Pol SM, Drexhage JA, van Het Hof B, Mebius R et al (2014) Astrocyte-derived retinoic acid: a novel regulator of blood-brain barrier function in multiple sclerosis. Acta Neuropathol 128(5):691–703
Mohajeri MH, Wollmer MA, Nitsch RM (2002) Abeta 42-induced increase in neprilysin is associated with prevention of amyloid plaque formation in vivo. J Biol Chem 277(38): 35460–35465
Mookherjee P, Green PS, Watson GS, Marques MA, Tanaka K, Meeker KD et al (2011) GLT-1 loss accelerates cognitive deficit onset in an Alzheimer's disease animal model. J Alzheimers Dis 26(3):447–455
Mosconi L (2013) Glucose metabolism in normal aging and Alzheimer's disease: methodological and physiological considerations for PET studies. Clin Transl Imaging 1:4. https://doi.org/10.1007/s40336-013-0026-y
Mosconi L, Mistur R, Switalski R, Tsui WH, Glodzik L, Li Y et al (2009) FDG-PET changes in brain glucose metabolism from normal cognition to pathologically verified Alzheimer's disease. Eur J Nucl Med Mol Imag 36(5):811–822
Mosconi L, Pupi A, De Leon MJ (2008) Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease. Ann N Y Acad Sci 1147:180–195
Mulder SD, Veerhuis R, Blankenstein MA, Nielsen HM (2012) The effect of amyloid associated proteins on the expression of genes involved in amyloid-beta clearance by adult human astrocytes. Exp Neurol 233(1):373–379

Murray ME, Przybelski SA, Lesnick TG, Liesinger AM, Spychalla A, Zhang B et al (2014) Early Alzheimer's disease neuropathology detected by proton MR spectroscopy. J Neurosci 34(49): 16247–16255

Nagele RG, Wegiel J, Venkataraman V, Imaki H, Wang KC (2004) Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. Neurobiol Aging 25(5):663–674

Nielsen HM, Mulder SD, Belien JA, Musters RJ, Eikelenboom P, Veerhuis R (2010) Astrocytic a beta 1-42 uptake is determined by a beta-aggregation state and the presence of amyloid-associated proteins. Glia 58(10):1235–1246

Nielsen HM, Veerhuis R, Holmqvist B, Janciauskiene S (2009) Binding and uptake of a beta1-42 by primary human astrocytes in vitro. Glia 57(9):978–988

Norris CM, Kadish I, Blalock EM, Chen KC, Thibault V, Porter NM, Landfield PW, Kraner SD (2005) Calcineurin triggers reactive/inflammatory processes in astrocytes and is upregulated in aging and Alzheimer's models. J Neurosci 25(18):4649–4658

Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B, Nedergaard M (2008) Loss of astrocytic domain organization in the epileptic brain. J Neurosci 28(13):3264–3276

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R et al (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39(3):409–421

Oh H, Madison C, Baker S, Rabinovici G, Jagust W (2016) Dynamic relationships between age, amyloid-beta deposition, and glucose metabolism link to the regional vulnerability to Alzheimer's disease. Brain 139(Pt 8):2275–2289

Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ (2010) Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. Glia 58(7):831–838

Olabarria M, Noristani HN, Verkhratsky A, Rodriguez JJ (2011) Age-dependent decrease in glutamine synthetase expression in the hippocampal astroglia of the triple transgenic Alzheimer's disease mouse model: mechanism for deficient glutamatergic transmission? Mol Neurodegen 6:55

Olsen M, Aguilar X, Sehlin D, Fang XT, Antoni G, Erlandsson A, Syvanen S (2018) Astroglial responses to amyloid-Beta progression in a mouse model of Alzheimer's disease. Mol Imaging Biol 20(4):605–614

Orellana JA, Shoji KF, Abudara V, Ezan P, Amigou E, Saez PJ, Jiang JX, Naus CC, Saez JC, Giaume C (2011) Amyloid beta-induced death in neurons involves glial and neuronal hemichannels. J Neurosci 31(13):4962–4977

Orre M, Kamphuis W, Osborn LM, Jansen AHP, Kooijman L, Bossers K, Hol EM (2014a) Isolation of glia from Alzheimer's mice reveals inflammation and dysfunction. Neurobiol Aging 35(12): 2746–2760

Orre M, Kamphuis W, Osborn LM, Melief J, Kooijman L, Huitinga I, Klooster J, Bossers K, Hol EM (2014b) Acute isolation and transcriptome characterization of cortical astrocytes and microglia from young and aged mice. Neurobiol Aging 35(1):1–14

Osborn LM, Kamphuis W, Wadman WJ, Hol EM (2016) Astrogliosis: an integral player in the pathogenesis of Alzheimer's disease. Prog Neurobiol 144:121–141

Palmer JC, Baig S, Kehoe PG, Love S (2009) Endothelin-converting enzyme-2 is increased in Alzheimer's disease and up-regulated by Abeta. Am J Pathol 175(1):262–270

Papadopoulos MC, Verkman AS (2013) Aquaporin water channels in the nervous system. Nat Rev Neurosci 14(4):265–277

Parker CA, Nabulsi N, Holden D, Lin SF, Cass T, Labaree D et al (2014) Evaluation of 11C-BU99008, a PET ligand for the imidazoline2 binding sites in rhesus brain. J Nucl Med 55(5):838–844

Parpura-Gill A, Beitz D, Uemura E (1997) The inhibitory effects of beta-amyloid on glutamate and glucose uptakes by cultured astrocytes. Brain Res 754(1–2):65–71

Patel NS, Paris D, Mathura V, Quadros AN, Crawford FC, Mullan MJ (2005) Inflammatory cytokine levels correlate with amyloid load in transgenic mouse models of Alzheimer's disease. J Neuroinflammation 2(1):9

Pekny M, Pekna M, Messing A, Steinhauser C, Lee JM, Parpura V, Hol EM, Sofroniew MV, Verkhratsky A (2016) Astrocytes: a central element in neurological diseases. Acta Neuropathol 131(3):323–345

Perez-Nievas BG, Serrano-Pozo A (2018) Deciphering the astrocyte reaction in Alzheimer's disease. Front Aging Neurosci 10:114

Peters O, Schipke CG, Philipps A, Haas B, Pannasch U, Wang LP, Benedetti B, Kingston AE, Kettenmann H (2009) Astrocyte function is modified by Alzheimer's disease-like pathology in aged mice. J Alzheimers Dis 18(1):177–189

Pihlaja R, Koistinaho J, Malm T, Sikkila H, Vainio S, Koistinaho M (2008) Transplanted astrocytes internalize deposited beta-amyloid peptides in a transgenic mouse model of Alzheimer's disease. Glia 56(2):154–163

Pitt J, Wilcox KC, Tortelli V, Diniz LP, Oliveira MS, Dobbins C et al (2017) Neuroprotective astrocyte-derived insulin/insulin-like growth factor 1 stimulates endocytic processing and extracellular release of neuron-bound Abeta oligomers. Mol Biol Cell 28(20):2623–2636

Porter JT, McCarthy KD (1996) Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. J Neurosci 16(16):5073–5081

Pow DV, Cook DG (2009) Neuronal expression of splice variants of "glial" glutamate transporters in brains afflicted by Alzheimer's disease: unmasking an intrinsic neuronal property. Neurochem Res 34(10):1748–1757

Pow DV, Crook DK (1996) Direct immunocytochemical evidence for the transfer of glutamine from glial cells to neurons: use of specific antibodies directed against the d-stereoisomers of glutamate and glutamine. Neuroscience 70(1):295–302

Prasad S, Sajja RK, Naik P, Cucullo L (2014) Diabetes mellitus and blood-brain barrier dysfunction: an overview. J Pharm 2(2):125

Priego N, Zhu L, Monteiro C, Mulders M, Wasilewski D, Bindeman W et al (2018) STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis. Nat Med 24(7):1024–1035

Querol-Vilaseca M, Colom-Cadena M, Pegueroles J, San Martin-Paniello C, Clarimon J, Belbin O, Fortea J, Lleo A (2017) YKL-40 (Chitinase 3-like I) is expressed in a subset of astrocytes in Alzheimer's disease and other tauopathies. J Neuroinflammation 14(1):118

Ransohoff RM (2016) How neuroinflammation contributes to neurodegeneration. Science 353(6301):777–783

Reichenbach N, Delekate A, Breithausen B, Keppler K, Poll S, Schulte T et al (2018) P2Y1 receptor blockade normalizes network dysfunction and cognition in an Alzheimer's disease model. J Exp Med 215(6):1649–1663

Ries M, Sastre M (2016) Mechanisms of Abeta clearance and degradation by glial cells. Front Aging Neurosci 8:160

Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, de la Monte SM (2005) Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. J Alzheimers Dis 8(3):247–268

Rodriguez JJ, Olabarria M, Chvatal A, Verkhratsky A (2009) Astroglia in dementia and Alzheimer's disease. Cell Death Differ 16(3):378–385

Rodriguez-Vieitez E, Ni R, Gulyas B, Toth M, Haggkvist J, Halldin C, Voytenko L, Marutle A, Nordberg A (2015) Astrocytosis precedes amyloid plaque deposition in Alzheimer APPswe transgenic mouse brain: a correlative positron emission tomography and in vitro imaging study. Eur J Nucl Med Mol Imaging 42(7):1119–1132

Rodriguez-Vieitez E, Saint-Aubert L, Carter SF, Almkvist O, Farid K, Scholl M et al (2016) Diverging longitudinal changes in astrocytosis and amyloid PET in autosomal dominant Alzheimer's disease. Brain 139(Pt 3):922–936

Rojas-Gutierrez E, Munoz-Arenas G, Trevino S, Espinosa B, Chavez R, Rojas K, Flores G, Diaz A, Guevara J (2017) Alzheimer's disease and metabolic syndrome: a link from oxidative stress and inflammation to neurodegeneration. Synapse 71(10):e21990

Rossner S, Lange-Dohna C, Zeitschel U, Perez-Polo JR (2005) Alzheimer's disease beta-secretase BACE1 is not a neuron-specific enzyme. J Neurochem 92(2):226–234

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE et al (2005) Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 433(7021):73–77

Rowan MS, Neymotin SA, Lytton WW (2014) Electrostimulation to reduce synaptic scaling driven progression of Alzheimer's disease. Front Comput Neurosci 8:39

Saha P, Sarkar S, Paidi RK, Biswas SC (2020) TIMP-1: a key cytokine released from activated astrocytes protects neurons and ameliorates cognitive behaviours in a rodent model of Alzheimer's disease. Brain Behav Immun 87:804–819

Samakashvili S, Ibanez C, Simo C, Gil-Bea FJ, Winblad B, Cedazo-Minguez A, Cifuentes A (2011) Analysis of chiral amino acids in cerebrospinal fluid samples linked to different stages of Alzheimer disease. Electrophoresis 32(19):2757–2764

Sarkar S, Biswas SC (2021) Astrocyte subtype-specific approach to Alzheimer's disease treatment. Neurochem Int 145:104956

Sastre M, Gentleman SM (2010) NSAIDs: how they work and their prospects as therapeutics in Alzheimer's disease. Front Aging Neurosci 2:20

Schallier A, Smolders I, Van Dam D, Loyens E, De Deyn PP, Michotte A, Michotte Y, Massie A (2011) Region- and age-specific changes in glutamate transport in the AbetaPP23 mouse model for Alzheimer's disease. J Alzheimers Dis 24(2):287–300

Schousboe A, Wellendorph P, Frolund B, Clausen RP, Krogsgaard-Larsen P (2017) Astrocytic GABA transporters: pharmacological properties and targets for antiepileptic drugs. Adv Neurobiol 16:283–296

Scimemi A, Meabon JS, Woltjer RL, Sullivan JM, Diamond JS, Cook DG (2013) Amyloid-beta1-42 slows clearance of synaptically released glutamate by mislocalizing astrocytic GLT-1. J Neurosci 33(12):5312–5318

Scott HA, Gebhardt FM, Mitrovic AD, Vandenberg RJ, Dodd PR (2011) Glutamate transporter variants reduce glutamate uptake in Alzheimer's disease. Neurobiol Aging 32:551

Seidel K, Vinet J, Dunnen WF, Brunt ER, Meister M, Boncoraglio A et al (2012) The HSPB8-BAG3 chaperone complex is upregulated in astrocytes in the human brain affected by protein aggregation diseases. Neuropathol Appl Neurobiol 38(1):39–53

Sekar S, McDonald J, Cuyugan L, Aldrich J, Kurdoglu A, Adkins J et al (2015) Alzheimer's disease is associated with altered expression of genes involved in immune response and mitochondrial processes in astrocytes. Neurobiol Aging 36(2):583–591

Selkoe DJ (2008) Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior. Behav Brain Res 192(1):106–113

Serrano-Pozo A, Mielke ML, Gomez-Isla T, Betensky RA, Growdon JH, Frosch MP, Hyman BT (2011) Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease. Am J Pathol 179(3):1373–1384

Sheldon AL, Robinson MB (2007) The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. Neurochem Int 51(6–7):333–355

Sheng JG, Bora SH, Xu G, Borchelt DR, Price DL, Koliatsos VE (2003) Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid beta peptide in APPswe transgenic mice. Neurobiol Dis 14(1):133–145

Shi Y, Yamada K, Liddelow SA, Smith ST, Zhao L, Luo W et al (2017) ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. Nature 549(7673):523–527

Simpson IA, Chundu KR, Davies-Hill T, Honer WG, Davies P (1994) Decreased concentrations of GLUT1 and GLUT3 glucose transporters in the brains of patients with Alzheimer's disease. Ann Neurol 35(5):546–551

Simpson JE, Fernando MS, Clark L, Ince PG, Matthews F, Forster G et al (2007) White matter lesions in an unselected cohort of the elderly: astrocytic, microglial and oligodendrocyte precursor cell responses. Neuropathol Appl Neurobiol 33(4):410–419

Simpson JE, Ince PG, Shaw PJ, Heath PR, Raman R, Garwood CJ et al (2011) Microarray analysis of the astrocyte transcriptome in the aging brain: relationship to Alzheimer's pathology and APOE genotype. Neurobiol Aging 32(10):1795–1807

Singh A, Abraham WC (2017) Astrocytes and synaptic plasticity in health and disease. Exp Brain Res 235(6):1645–1655

Singh D, Agrawal A, Singal CMS, Pandey HS, Seth P, Sharma SK (2020) Sinomenine inhibits amyloid beta-induced astrocyte activation and protects neurons against indirect toxicity. Mol Brain 13(1):30

Sirko S, Behrendt G, Johansson PA, Tripathi P, Costa M, Bek S et al (2013) Reactive glia in the injured brain acquire stem cell properties in response to sonic hedgehog. [corrected]. Cell Stem Cell 12(4):426–439

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32(12):638–647

Sofroniew MV (2014a) Astrogliosis. Cold Spring Harb Perspect Biol 7(2):a020420

Sofroniew MV (2014b) Multiple roles for astrocytes as effectors of cytokines and inflammatory mediators. Neuroscientist 20(2):160–172

Sofroniew MV (2015) Astrocyte barriers to neurotoxic inflammation. Nat Rev Neurosci 16(5): 249–263

Sollvander S, Nikitidou E, Brolin R, Soderberg L, Sehlin D, Lannfelt L, Erlandsson A (2016) Accumulation of amyloid-beta by astrocytes result in enlarged endosomes and microvesicle-induced apoptosis of neurons. Mol Neurodegen 11(1):38

Soucek T, Cumming R, Dargusch R, Maher P, Schubert D (2003) The regulation of glucose metabolism by HIF-1 mediates a neuroprotective response to amyloid beta peptide. Neuron 39(1):43–56

Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X, Nedergaard M (2006) Astrocyte-mediated control of cerebral blood flow. Nat Neurosci 9(2):260–267

Takeda S, Sato N, Morishita R (2014) Systemic inflammation, blood-brain barrier vulnerability and cognitive/non-cognitive symptoms in Alzheimer disease: relevance to pathogenesis and therapy. Front Aging Neurosci 6:171

Talantova M, Sanz-Blasco S, Zhang X, Xia P, Akhtar MW, Okamoto S et al (2013) Abeta induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. Proc Natl Acad Sci U S A 110(27):E2518–E2527

Terai K, Iwai A, Kawabata S, Sasamata M, Miyata K, Yamaguchi T (2001) Apolipoprotein E deposition and astrogliosis are associated with maturation of beta-amyloid plaques in betaAPPswe transgenic mouse: implications for the pathogenesis of Alzheimer's disease. Brain Res 900(1):48–56

Thal DR, Schultz C, Dehghani F, Yamaguchi H, Braak H, Braak E (2000) Amyloid beta-protein (Abeta)-containing astrocytes are located preferentially near N-terminal-truncated Abeta deposits in the human entorhinal cortex. Acta Neuropathol 100(6):608–617

Tomimoto H, Akiguchi I, Suenaga T, Nishimura M, Wakita H, Nakamura S, Kimura J (1996) Alterations of the blood-brain barrier and glial cells in white-matter lesions in cerebrovascular and Alzheimer's disease patients. Stroke 27(11):2069–2074

Tyacke RJ, Fisher A, Robinson ES, Grundt P, Turner EM, Husbands SM, Hudson AL, Parker CA, Nutt DJ (2012) Evaluation and initial in vitro and ex vivo characterization of the potential positron emission tomography ligand, BU99008 (2-(4,5-dihydro-1H-imidazol-2-yl)-1- methyl-1H-indole), for the imidazoline(2) binding site. Synapse 66(6):542–551

Tyzack GE, Sitnikov S, Barson D, Adams-Carr KL, Lau NK, Kwok JC et al (2014) Astrocyte response to motor neuron injury promotes structural synaptic plasticity via STAT3-regulated TSP-1 expression. Nat Commun 5:4294

Verghese PB, Castellano JM, Garai K, Wang Y, Jiang H, Shah A, Bu G, Frieden C, Holtzman DM (2013) ApoE influences amyloid-beta (Abeta) clearance despite minimal apoE/Abeta association in physiological conditions. Proc Natl Acad Sci U S A 110(19):E1807–E1816

Verkhratsky A, Parpura V, Rodriguez-Arellano JJ, Zorec R (2019a) Astroglia in Alzheimer's disease. In: Verkhratsky A, Ho M, Zorec R, Parpura V (eds) Neuroglia in neurodegenerative diseases. Springer, Singapore

Verkhratsky A, Rodrigues JJ, Pivoriunas A, Zorec R, Semyanov A (2019b) Astroglial atrophy in Alzheimer's disease. Pflugers Arch 471(10):1247–1261

Verkhratsky A, Zorec R, Parpura V (2017) Stratification of astrocytes in healthy and diseased brain. Brain Pathol 27(5):629–644

Verkhratsky A, Zorec R, Rodriguez JJ, Parpura V (2016) Astroglia dynamics in ageing and Alzheimer's disease. Curr Opin Pharmacol 26:74–79

Walls AB, Waagepetersen HS, Bak LK, Schousboe A, Sonnewald U (2015) The glutamine-glutamate/GABA cycle: function, regional differences in glutamate and GABA production and effects of interference with GABA metabolism. Neurochem Res 40(2):402–409

Wang D, Zhang X, Wang M, Zhou D, Pan H, Shu Q, Sun B (2018) Early activation of astrocytes does not affect amyloid plaque load in an animal model of Alzheimer's disease. Neurosci Bull 34(6):912–920

Wang Q, Wu J, Rowan MJ, Anwyl R (2005) Beta-amyloid inhibition of long-term potentiation is mediated via tumor necrosis factor. Eur J Neurosci 22(11):2827–2832

Woltjer RL, Duerson K, Fullmer JM, Mookherjee P, Ryan AM, Montine TJ et al (2010) Aberrant detergent-insoluble excitatory amino acid transporter 2 accumulates in Alzheimer disease. J Neuropathol Exp Neurol 69(7):667–676

Wu HY, Hudry E, Hashimoto T, Kuchibhotla K, Rozkalne A, Fan Z et al (2010) Amyloid beta induces the morphological neurodegenerative triad of spine loss, dendritic simplification, and neuritic dystrophies through calcineurin activation. J Neurosci 30(7):2636–2649

Wu Z, Guo Z, Gearing M, Chen G (2014) Tonic inhibition in dentate gyrus impairs long-term potentiation and memory in an Alzheimer's [corrected] disease model. Nat Commun 5:4159

Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J (2003) Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. Nat Med 9(4):453–457

Xiao Q, Yan P, Ma X, Liu H, Perez R, Zhu A et al (2014) Enhancing astrocytic lysosome biogenesis facilitates Abeta clearance and attenuates amyloid plaque pathogenesis. J Neurosci 34(29): 9607–9620

Yamaguchi H, Sugihara S, Ogawa A, Saido TC, Ihara Y (1998) Diffuse plaques associated with astroglial amyloid beta protein, possibly showing a disappearing stage of senile plaques. Acta Neuropathol 95(3):217–222

Yamamoto M, Kiyota T, Horiba M, Buescher JL, Walsh SM, Gendelman HE, Ikezu T (2007) Interferon-gamma and tumor necrosis factor-alpha regulate amyloid-beta plaque deposition and beta-secretase expression in Swedish mutant APP transgenic mice. Am J Pathol 170(2):680–692

Yang J, Zhang R, Shi C, Mao C, Yang Z, Suo Z, Torp R, Xu Y (2017) AQP4 association with amyloid deposition and astrocyte pathology in the Tg-ArcSwe mouse model of Alzheimer's disease. J Alzheimers Dis 57(1):157–169

Yao J, Rettberg JR, Klosinski LP, Cadenas E, Brinton RD (2011) Shift in brain metabolism in late onset Alzheimer's disease: implications for biomarkers and therapeutic interventions. Mol Aspects Med 32(4–6):247–257

Yin KJ, Cirrito JR, Yan P, Hu X, Xiao Q, Pan X et al (2006) Matrix metalloproteinases expressed by astrocytes mediate extracellular amyloid-beta peptide catabolism. J Neurosci 26(43): 10939–10948

Yoshiike Y, Kimura T, Yamashita S, Furudate H, Mizoroki T, Murayama M, Takashima A (2008) GABA(a) receptor-mediated acceleration of aging-associated memory decline in APP/PS1 mice and its pharmacological treatment by picrotoxin. PLoS One 3(8):e3029

Yun SP, Kam TI, Panicker N, Kim S, Oh Y, Park JS et al (2018) Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. Nat Med 24(7):931–938

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32(18):6391–6410

Zhao J, O'Connor T, Vassar R (2011) The contribution of activated astrocytes to Abeta production: implications for Alzheimer's disease pathogenesis. J Neuroinflammation 8:150

Zhou Y, Song WM, Andhey PS, Swain A, Levy T, Miller KR et al (2020) Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. Nat Med 26(1):131–142

Zimmer ER, Parent MJ, Souza DG, Leuzy A, Lecrux C, Kim HI et al (2017) [(18)F]FDG PET signal is driven by astroglial glutamate transport. Nat Neurosci 20(3):393–395

Zulfiqar S, Garg P, Nieweg K (2019) Contribution of astrocytes to metabolic dysfunction in the Alzheimer's disease brain. Biol Chem 400(9):1113–1127

Zumkehr J, Rodriguez-Ortiz CJ, Cheng D, Kieu Z, Wai T, Hawkins C et al (2015) Ceftriaxone ameliorates tau pathology and cognitive decline via restoration of glial glutamate transporter in a mouse model of Alzheimer's disease. Neurobiol Aging 36(7):2260–2271

Role of Astrocyte Dysfunction in Parkinson's Disease Pathogenesis

Indrani Datta, Aishwarya Raj, and Alka Kaushal

Abstract

Astrocytes are the major glial constituents of the central nervous system and are critical for brain function. Despite this, it is relatively recently that researchers have started considering these niche cells for possible involvement in neurodegenerative diseases such as Parkinson's disease (PD). PD is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). While neuronal Lewy Body inclusions in dopaminergic neurons comprising mainly of α-synuclein serves as a pathological hallmark of PD, recent studies have shown α-synuclein immunoreactive inclusions even in astrocytes of PD patient brains. Several studies have also reported that genes known to have a causative role in the development of PD are expressed in astrocytes and have important roles in astrocyte function. Astrocytes are multifunctional cells contributing to the survival and maintenance of neuronal health, ion buffering, neurotransmitter recycling, and regulation of the blood-brain barrier. Consequences of the loss of normal homeostatic functions and an increase in toxic functions in astrocytes are thus probably involved in the onset and progression of PD. Further, the complex role of astrocytes is influenced by region-specificity and the number of astrocytes for the survival of dopaminergic neurons. Indeed, studies have demonstrated regional variability in gene expression of astrocytes of the cortex, cerebellum, brainstem, and hypothalamus. These emerging roles of astrocytes in the pathogenesis of PD constitute an exciting development with promising novel therapeutic targets to modify the hostile microenvironment of substantia nigra during PD. Understanding these niche cells is also of prime importance for designing approaches for prophylactic and regenerative strategies through

I. Datta (✉) · A. Raj · A. Kaushal
Department of Biophysics, National Institute of Mental Health and Neurosciences, Institute of National Importance, Bengaluru, Karnataka, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_10

derivation of exogenous niche cells from patient-specific iPSCs or mesenchymal stromal cells. Here, we review the potential protective and deleterious effects of astrocytes in the substantia nigra of PD and explore how recent developments can in turn impact our understanding of the pathophysiology of PD and its treatment.

Keywords

Parkinson's disease · Astrocytes · α-Synuclein · Heterogeneity · Dopaminergic neurons

1 Introduction

PD (Griffiths et al. 1999) is a progressive neurodegenerative disorder that is marked primarily by the specific loss of DA neurons in the SNpc (Desai Bradaric et al. 2012) region of the brain. Pathologically, it is recognized by the presence of intracellular protein aggregates known as LBs constituted of α-synuclein (Auluck et al. 2010). The main clinical symptoms are bradykinesia, rigidity, and tremors, and these motor symptoms emerge when striatal dopamine levels come down to a level of 70–80%, due to the death of the nigral DA neurons. The etiology and pathogenesis of PD are highly complex involving both environmental and genetic factors. Studies on genetic mutations associated with early-onset and sporadic PD have revealed the involvement of a few genes. The autosomal dominant genes for encoding α-synuclein (Kim et al. 2016), LRRK2 (Angeles et al. 2011; Zimprich et al. 2004), and autosomal recessive genes PARK2 (Auluck et al. 2010); PARK7 (Bonifati et al. 2003); PINK1 (previously PARK6); and ATP13A2 (previously PARK9) have provided significant apprehension about DA neuronal death, which include neuroinflammation, oxidative stress, mitochondrial dysfunction, and insufficient autophagic or proteasomal protein degradation (Booth et al. 2017). Among environmental factors, exposure to heavy metals, pesticides, and herbicides are shown to be associated with PD. Over the last 40 years, dopamine replacement therapy is the prevalent mode of treatment, and it is well-known that its long-term usage leads to serious cognitive and metabolic side effects.

A distinctive pathophysiological feature of the disease is the abnormal accumulation of the misfolded protein α-synuclein—the predominant protein found in LBs (Gibb and Lees 1988). Despite not knowing the exact physiological role of α-synuclein yet, it is considered to play role in the formation and maintenance of regulation of lipid metabolism, synaptic vesicle pools, and calcium signaling in DA neurons (Auluck et al. 2010; Totterdell and Meredith 2005). In normal conditions, α-synuclein exists in monomeric form and is recognized and cleared through the ubiquitin-proteasome system (Bernhard et al. 2016) and CMA (Giaume et al. 2010) pathways (Mazzulli et al. 2016). Misfolding or mutations of α-synuclein (A30P/A53T) in a diseased condition changes its monomeric form into aggregated state forming LBs in the DA neurons (Janda et al. 2012). This aberrant level of α-synuclein is seen in familial as well as idiopathic PD subjects (Liu et al. 2018).

Earlier, α-synuclein was believed to exert its pathogenic effects through its intracellular (Parnetti et al. 2016; Tokuda et al. 2010; Wang et al. 2018) localization, but recent studies (Lee et al. 2006) have detected it in human plasma and CSF (Mashayekhi et al. 2010) as well. The transfer of this exocytosed α-synuclein to other neurons is common, and their adverse effects on the neurons have been extensively studied (Desplats et al. 2009; Freeman et al. 2013; Sung et al. 2005).

However, α-synuclein aggregation is not limited to DA neuronal cell bodies but also appears commonly in astrocytes at advanced stages of the disease (Braak et al. 2007; Croisier et al. 2006; Terada et al. 2003; Tu et al. 1998; Wakabayashi et al. 2000). Astrocytes, the most abundant glia of the mammalian brain, exceed neurons in number by several-fold in the human brain (Sofroniew and Vinters 2010). These cells are in close proximity with other neuronal and non-neuronal components of the brain, thereby placing itself in a strategic position to modulate multiple functions in the neural tissue. The earlier typecast of astrocytes as a kind of glue that is responsible for mere physical/mechanical support to hold the neurons in place is being increasingly revised with the acknowledgement that these cells actively play critical and vital roles in facilitating the physiologic and pathologic states of neurons (Barres 2008; Farina et al. 2007). Historically, astrocytes were not being considered for their possible involvement in neurodegenerative diseases such as PD. Of late, however, several studies have reported that genes known to have a causative role in PD are expressed in astrocytes and have significant roles in their function (Booth et al. 2017), suggesting that dysfunction of astrocytes may be related to PD development. We will address these issues in this chapter.

Astrocytes constitute the microenvironment of neurons and can make it either neuroprotective or neurodegenerative, largely depending on the molecules that they release and pick-up from the extracellular space. For instance, secretion of NFs such as NT-3, nerve growth factor (Pei et al. 2019) and basic fibroblast growth factor (Datta et al. 2011), astrocytes also supply neurons with metabolic substrates such as lactate and the antioxidant GSH (Gegg et al. 2003) that play an important role in the survival and appropriate functioning of neurons. Additionally, astrocytes confer neuroprotection by drawing off surplus extracellular excitotoxic agents, such as glutamate, potassium, and calcium. On the other hand, when astrocytes endure a state of gliosis in response to neuronal damage or toxic insults, they (Desai Bradaric et al. 2012) release cytokines and chemokines that are deleterious to neurons. Here, we will not only discuss how astrocytes play a role as the DA neuron niche but also summarize recent studies on the dual role of astrocytes in neuronal survival and function in the context of PD. The chapter will also discuss the scientific findings regarding the heterogeneity of astrocytes concerning number and region specificity, as well as their multiple physiological functions.

2 Astrocytes: Role as Forming the Niche for DA Neurons

DA neurons are known to be more vulnerable than other neuronal cells to oxidative stress due to the intrinsic properties of the neurotransmitter dopamine and the presence of iron. In DA cells, deamination of dopamine by MAO (Tokuda et al. 2010) results in significant yields of H_2O_2 (Solano et al. 2008b) that can interact further with the reduced forms of transition metal ions such as iron and decompose to form highly reactive hydroxyl radicals (Carvey et al. 2005). Autopsy studies have reported elevated levels of iron in the SNpc region of PD patients brain compared to healthy individuals (Dexter et al. 1989; Gerlach et al. 1994; Griffiths et al. 1999). Unlike healthy persons, the increase in ROS does not automatically enhance the production of antioxidants in PD patients. This could be because PD is an age-associated disorder and the activity of cellular detoxification enzymes, such as SOD (Hawkins and Butt 2013) and GPx (Liddell et al. 2006), naturally declines with age (Venkateshappa et al. 2012).

Under physiological conditions, the microenvironment of the DA neurons is tightly maintained so that the terminally differentiated DA neurons not only survive but are also capable of functioning efficiently (Fig. 1). This balance is maintained largely by the niche cells, the astrocytes. As shown in Fig. 1, astrocytes make contact with both capillaries and neurons in the CNS (Argaw et al. 2012). For proper

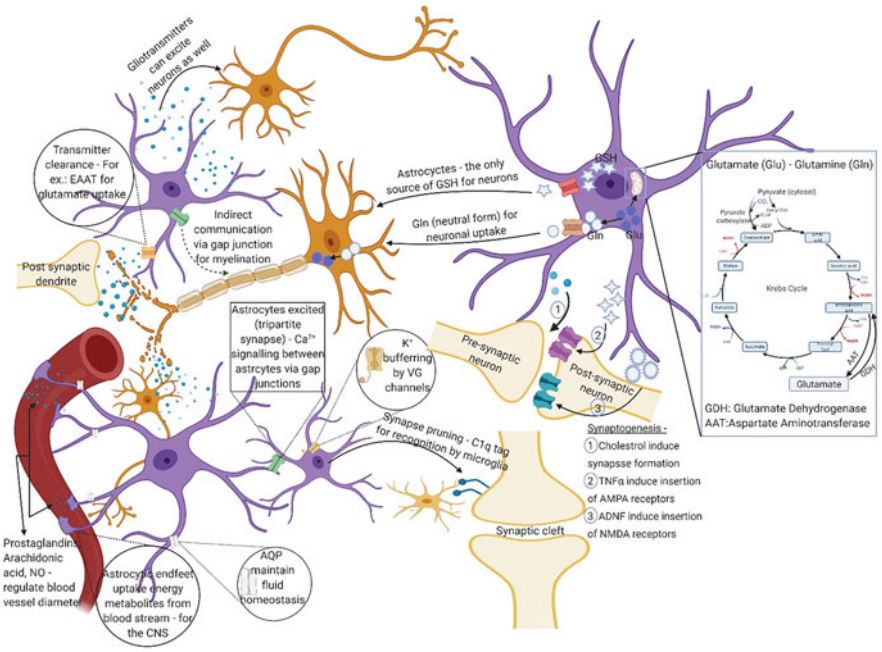

Fig. 1 Multiple functions of astrocytes as niche cells for optimal maintenance, survival, and function of DA neurons. (Figure was prepared using BioRender (https://biorender.com/))

survival of DA neurons, astrocytes provide nutrients such as NFs and antioxidant substrate GSH (Joe et al. 2018; Raps et al. 1989; Tsacopoulos and Magistretti 1996).

GSH is produced intracellularly by γ-glutamylcysteine synthetase and glutathione synthetase. The GSH concentration in astrocytes (~3.8 mmol/L) is assessed to be greater than that of neurons (~2.5 mmol/L) (Bolanos et al. 1995; Rappold and Tieu 2010; Rice and Russo-Menna 1997) because of the better specific activity of the γ-glutamylcysteine synthetase in astrocytes (Gegg et al. 2003). Because of their proximity, astrocytes can share their GSH with the adjacent neurons by releasing this antioxidant substrate through the MRP1 transporter into the extracellular space (Hirrlinger et al. 2002b). Further, GSH is cleaved by γ-glutamyl-transpeptidase on the astrocytic plasma membrane to produce precursors for neuronal GSH synthesis. This GSH release seems to be a unique activity of astrocytes and not demonstrated by neurons, microglia, and oligodendrocytes (Hirrlinger et al. 2002b), probably due to decreased levels of MRP1 in these cell types (Hirrlinger et al. 2002a). Consistent with the neuroprotective role of GSH, decreases in GSH levels have been reported in in vivo models and even in patients suffering from PD (Zeevalk et al. 2008). The GSH content in the SN of PD patients is significantly reduced (~40%) (Sian et al. 1994), and at the cellular level, significant loss of GSH is reported in surviving nigral DA neurons (Pearce et al. 1997). In genetic models of PD, astrocytes aged in cultures from parkin-KO (Solano et al. 2008b) mice show lower levels of GSH than those from WT (Argaw et al. 2012) animals (Solano et al. 2008b). However clinical trial of parenteral GSH administration in PD patients failed to show any benefits (Hauser et al. 2009) which could be due to the low permeability of GSH across the BBB (Argaw et al. 2012; Cornford et al. 1978; Kannan et al. 1990; McLellan et al. 1995).

Astrocytes secrete a variety of NFs such as bFGF (Grothe and Timmer 2007), GDNF (Saavedra et al. 2006) and MANF (Petrova et al. 2004). Among these trophic factors, GDNF has been most widely researched and has been found to confer maximum protection of DA neurons in pre-clinical models of PD (Deierborg et al. 2008). However, the clinical trials data was not encouraging due to side effects and conflicting results (Lang et al. 2006b). Here too, the permeability of these factors across BBB is a major limitation.

Astrocytes also maintain homeostasis of the brain microenvironment through uptake of extracellular glutamate via the EAAT-1/2 and potassium ions through the inward-rectifier potassium channel, Kir4.1, in addition to immunomodulation. They also maintain the extracellular water content through AQP4 and are the primary cells responsible for glucose metabolism regulation in the brain. Due to their anatomical location of lining the BBB, they regulate the formation of the BBB, modulate the tone of blood vessels (Lee et al. 2003; Li et al. 2008; Mulligan and MacVicar 2004), and are the main cells in CNS that take up glucose from the blood and use glycolysis to supply energy to neurons in the form of lactate (Tsacopoulos and Magistretti 1996). Astrocytes also store glucose as glycogen (Brown 2004; Vilchez et al. 2007). In addition, they convert glutamate to glutamine by the action of glutamine synthetase and provide neurons with glutamine (Norenberg and Martinez-Hernandez 1979; Ramaharobandro et al. 1982), which the neurons then convert into glutamate for neurotransmission. Astrocytes are also reported to have

cross talk with neurons through the release of gliotransmitters including GABA and glutamate (Lee et al. 2010a; Newman 2003). Further astrocytes are known for the ability of the glymphatic system (the equivalent of the lymphatic system elsewhere in the blood) to eliminate waste in the brain (Iliff and Nedergaard 2013) and regulate the formation and/or phagocytosis of synapses (Chung et al. 2013; Ronnevi 1978). Thus, through myriad and multifunctional ways, astrocytes play the overall role of niche cells that regulate the microenvironment of the neurons.

There are distinct functions of astrocytes that are specific to the midbrain, in comparison to other CNS regions (Boisvert et al. 2018; Chever et al. 2014; Gosejacob et al. 2011). For example, the requirement of increased potassium buffering capacity, greater metabolic support, and sensitivity to dopamine signaling for the tonic firing of DA neurons, as a way of gauging DA neuron health and function. Therefore, the role of niche cells or astrocytes become extremely important in PD.

3 Astrocytes and Their Heterogeneity and Region Specificity

Ontogenic development of glial cells has highlighted two crucial aspects of astrocytes that perhaps play a vital role in neurodegenerative diseases—their heterogeneity for different regions of the brain and (Gibb and Lees 1988) their number (Chever et al. 2014; D'Ambrosio et al. 1998; Djukic et al. 2007; Giaume et al. 2010; Gosejacob et al. 2011; Itoh et al. 2018; Lin et al. 1998). While astrocytes have a lot in common across different regions of the brain, it is increasingly evident that they exhibit functional and molecular heterogeneity (Chaboub and Deneen 2012) in a region-dependent manner (Bayraktar et al. 2015; Datta et al. 2018; Zhang and Barres 2010). Structural heterogeneity is observed among astrocytes from different regions.

Recent studies provide convincing evidence of regional astrocyte heterogeneity, which likely stems from adaptation to the requirement of the local neuronal population and from developmentally programmed differences (Datta et al. 2018; Farhy-Tselnicker et al. 2017; Fiacco and McCarthy 2018). Functional differences were reported among astrocytes isolated from postnatal day 1–3 rat forebrain, midbrain, and hindbrain in rendering neuroprotection to DA neuronal cells under 6-OHDA (Datta et al. 2018) stress, and this was mediated through differential BDNF release (Datta et al. 2018). Xin et al. (2019) too reported that ventral midbrain astrocytes are physiologically distinct from astrocytes in the cortex and hippocampus. Ventral midbrain astrocytes showed very low membrane resistance and inward-rectifying potassium channel-mediated current and were extensively coupled to surrounding oligodendrocytes through gap junctions. They exhibited calcium responses to glutamate but were relatively insensitive to norepinephrine, and responded to DRD2 signaling. Transcriptional differences between two closely related areas in the midbrain—SN and VTA—were reported (Kostuk et al. 2018), demonstrating further subregional heterogeneity. GDF15, a member of the TGFβ superfamily, was found to be expressed 230-fold higher in VTA astrocytes than SN, probably leading to higher neuroprotection.

In respect to PD, the midbrain region is crucial and cues from this region to astrocytes may be of prime importance in describing the disease pathology. Studies have evidenced the presence of reactive astrocytes containing α-synuclein in the striatum and dorsal thalamus (Braak et al. 2007), suggesting that astrocytes specific to these regions may play a key role in PD development. Factors, such as bFGF, GDNF, BDNF, etc., released by astrocytes from different regions of the brain can selectively mediate differential neuron responsiveness and functionality. In this context, increased BDNF is required for the neuroprotection of midbrain neurons, as shown in conditions mimicking PD (Datta et al. 2018). Furthermore, it is known that there can be many age-related changes in the gene expression profile of astrocytes in the SN region, again indicating a connection with PD with its selective neurodegeneration in the SN. Controlling the reactivity of astrocytes can reduce the neurodegeneration of DA neurons in these regions.

Glutamate is one of the important gliotransmitters, secreted by the astrocytes in response to certain stimuli. This activity of an astrocyte is also heterogeneous, as evidenced by several studies (Martin et al. 2015). In the striatum, astrocytes can efficiently differentiate between DA neurons expressing DRD1 and DRD2. When these astrocytes are stimulated by either of these sets of neurons by the release of endocannabinoids, a calcium spike is observed in the respective population of astrocytes leading to glutamate release that can activate the neurons expressing DRD1 or DRD2 as needed (Martin et al. 2015). With glutamate release, the effects of glutamate excitotoxicity caused by dystrophic niche astrocytes surrounding DA neurons in the pathophysiology of PD must also be considered.

Astrocyte number is the other key parameter that has an impact on neuronal health. Even though they reside in the same general mesencephalon region, post-mortem analysis of PD patient brains shows that DA neurons in the VTA do not degenerate unlike those in the SN (Brichta and Greengard 2014; Fu et al. 2016). The main difference between these two regions of the midbrain both of which have DA neurons is the number of astrocytes. Rappold and Tieu (2010) have shown that the survival of DA neurons is dependent on the presence of astrocytes. The influence of the number of astrocytes on the survival of DA neurons under oxidative stress is reported by Datta et al. (2018) in an in vitro model. In this study, purified astrocytes were acquired from forebrain, midbrain, and hindbrain regions and were characterized through flow cytometry and IF. The authors then showed that cell survival was augmented with an increase in astrocyte seeding number. While total cell survival was comparable among the different region-specific astrocytes for all numbers, striking differences were observed in DA neuronal (TH positive) cell survival in the presence of midbrain astrocytes in comparison to forebrain and hindbrain astrocytes, thereby emphasizing regional heterogeneity among astrocytes.

Table 1 Genes associated with PD

Gene	Loci chromosomes	Proteins	Forms of PD and age	Onset references
PARK-7	1p36	DJ-1	Early-onset autosomal recessive PD	Bonifati et al. (2003), Choi et al. (2018), Junn et al. (2005), Mullett and Hinkle (2009)
SNCA	4q22.1	α-Synuclein	Early-onset of familial PD Mutations or duplication of the gene	Solano et al. (2000)
LRRK2	12q12	LRRK2	Autosomal dominant parkinsonism presents a phenotype similar to idiopathic PD	
PARKIN	78C2-78C2	Parkin	Autosomal recessive form of PD Genetic recessive PD	
PLA2G6	22q13.1	iPLA2β	Autosomal recessive early-onset PD Neurodegeneration with brain iron accumulation	Kinghorna and Castillo-Quana (2016), Lin et al. (2018), Ramanadham et al. (2015)
ATP13A2	1p36.13	ATP13A	Autosomal recessive early-onset PD Decrease in mitochondrial membrane potential, increase in ROS production, mitochondrial fragmentation and ATP depletion	Park et al. (2014, 2015)
GBA	1q21-22	GCase	Autosomal-recessive, late-onset The genetic risk factor associated with Parkinsonism	Velayati et al. (2010)
PINK1	1p36.12	PINK1	Early-onset PD Loss-of-function mutations, mitochondrial dysfunction, and decreased proliferation of astrocytes	Kawajiri et al. (2011)

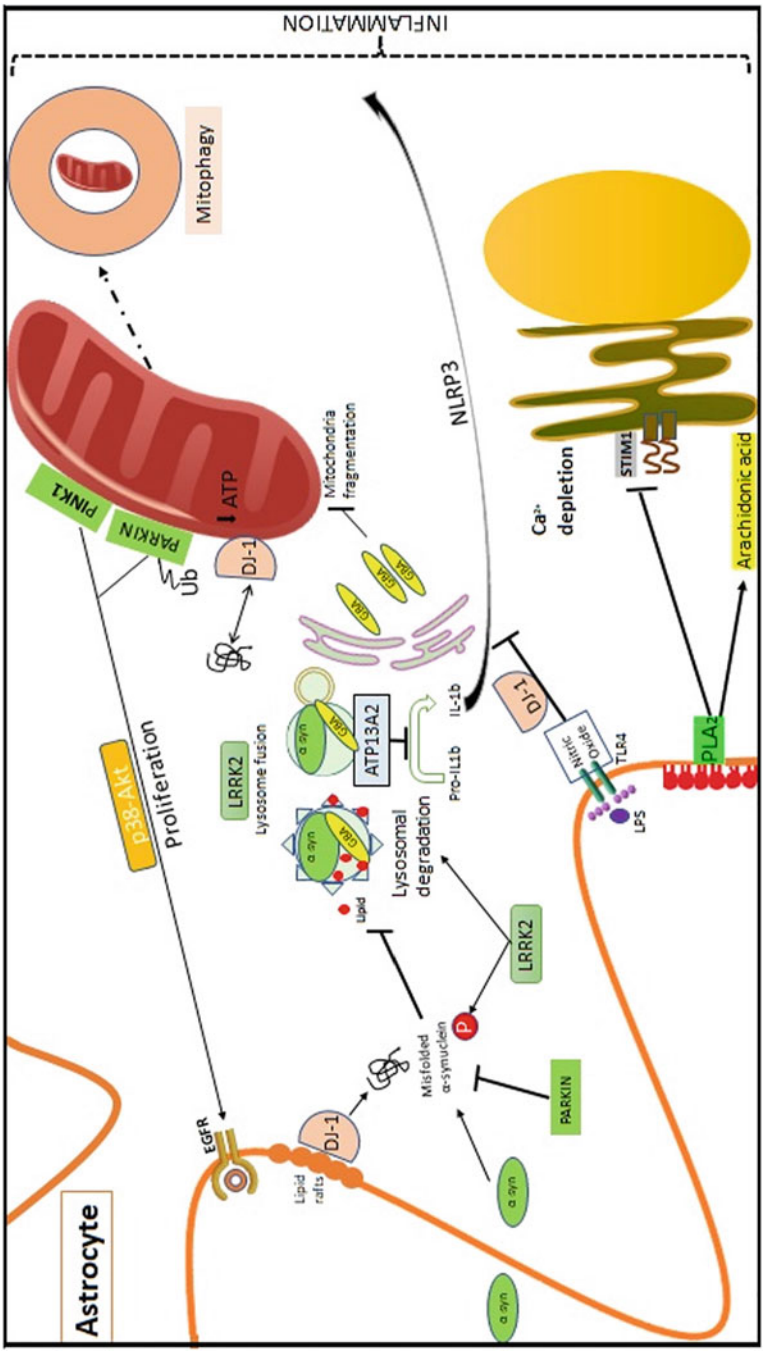

Fig. 2 The PD associated genes about their organellar localization and impairment of astrocytes. (Figure was prepared using BioRender (https://biorender.com/))

4 PD-Related Genes Associated with Astrocytes (PARK-7, SNCA, LRRK2, PARK-2, PLA2G6, ATP13A2, GBA, PINK1)

Table 1 lists the genes associated with PD, and Fig. 2 represents the PD-related genes associated with astrocytes.

1. **PARK-7** is an early-onset autosomal recessive PD associated gene that codes for the DJ-1 protein and is known to mediate neuroprotection by regulating apoptosis, oxidative stress, and astrogliosis in PD. Mutations in DJ-1 have been associated with PD (Bonifati et al. 2003; Choi et al. 2018; Junn et al. 2005; Mullett and Hinkle 2009). Postmortem analysis of human PD patients has shown higher expression of DJ-1 in astrocytes in comparison to neurons (Bandopadhyay et al. 2004). Animal studies have also shown a similar elevated astrocytic expression of DJ-1 in the hippocampal and cortical cultures of mouse brain (Ramsey et al. 2010). The upregulation of DJ-1 in reactive astrocytes identifies its role as a regulator of proinflammatory responses. In a study on DJ-1 KO astrocytes, LPS treatment generated >10 times more NO than littermate controls (Waak et al. 2009). This LPS-led NO production was mediated by the ROS signaling pathway, which coincided with the induction of proinflammatory mediator's COX-2 and IL-6 in DJ-1 KO astrocytes (Kim et al. 2016). However, on DJ-1 lentiviral transduction in astrocytes, the LPS-induced production of NO was significantly reduced, suggesting that the PD pathogenesis by deregulation of astrocytic neuroinflammatory damage was contributed by loss of DJ-1 (Waak et al. 2009).

   In astrocytes, DJ-1 is shown to interact with lipid rafts, influencing membrane receptor trafficking, endocytosis, and signal transduction. Inactivation of DJ-1 impaired lipid rafts-dependent signaling pathways leads to dysfunction of astrocytes, which may contribute to DA neuron degeneration in the SNpc (Kim et al. 2013). It was also seen that LPS treatment of primary astrocytes resulted in the localization of DJ-1 into lipid rafts. DJ-1 KO mice presented augmented LPS-TLR-4 signaling in primary astrocytes, further confirming the role of DJ-1 in lipid rafts-dependent endocytosis, which may cause the neurodegeneration seen in PD. DJ-1-deficient primary astrocytes induced alteration in lipid rafts-dependent endocytosis through the impairment of expression of main protein components of lipid rafts, flotillin-1, and caveolin-1 (Kim et al. 2016).

   Furthermore, DJ-1 deficiency also affected astrocytic glutamate uptake by altering the expression of glutamate transporter EAAT2 (Su et al. 2003). Overexpression of DJ-1 in astrocytes showed increased neuroprotection against the neurotoxin rotenone in neuron-astrocyte contact and noncontact co-cultures. Further, rotenone treatment of DJ-1 KO astrocytes impaired their protective role when compared to WT astrocytes (Mullett and Hinkle 2009). The DJ-1 KO attenuates the ability of astrocytes to support neuronal cells which were further confirmed in an in vivo model of 6-OHDA-induced PD. Astrocytes isolated from DJ-1 KO mice failed to protect human neuroblastoma cells against 6-OHDA treatment as compared to WT astrocytes (Lev et al. 2013). These studies confirm that loss of DJ-1 in astrocytes impaired its neuroprotection in PD. In addition, DJ-1 has been found to maintain mitochondria within astrocytes. Knockdown of

Park-7 decreased astrocytic mitochondrial motility in the same mode as rotenone treatment, and this knockdown exacerbated the decrease in astrocyte mitochondrial membrane potential caused by rotenone treatment (Mullett and Hinkle 2009).

2. **SNCA** encodes for an α-synuclein protein (Cabezas et al. 2014), and mutations or duplication of the gene have been linked to the development of PD. Expression of SNCA is predominantly neuronal, with very low expression observed in astrocytes (Solano et al. 2000). However, in postmortem PD brains, α-synuclein-positive inclusions were also seen in astrocytes along with neurons, which depicts PD pathology parallel with the formation of cortical intraneuronal Lewy neurites and LBs (Braak et al. 2007). The presence of astrocytic α-synuclein could be because the excess α-synuclein secreted by neurons is taken up by astrocytes in PD via TLR4-independent endocytosis pathway that localizes α-synuclein to lysosomes for degradation (Rannikko et al. 2015). The accumulation of α-synuclein in astrocytes beyond a certain threshold disrupts their physiological functions of maintaining CNS homeostasis and BBB integrity (Cabezas et al. 2014). A high concentration of extracellular α-synuclein, when not degraded by astrocytes, activates an inflammatory response that contributes to PD pathology (Lee et al. 2010b). The same was depicted by overexpressing mutant SNCA under an astrocyte-specific promoter in a mouse model, where α-synuclein over accumulation resulted in astrogliosis followed by neurodegeneration and motor impairment. Such mice showed impaired expression of GLAST1 and GLT1 along with BBB dysfunction due to abnormal localization of water channel AQP4 (Lee et al. 2017; Mader and Brimberg 2019). All these pathological changes then contributed to DA neuron degeneration in SNpc (60.5%) and VTA (26.1%).

   In another α-synuclein toxicity study conducted on human astrocytes, mitochondrial dysfunction was found to be activated leading to cellular degeneration and cell death (Braidy et al. 2013). Astrocytes play a crucial role in fatty acid metabolism in the brain, as is evident from a study done on SNCA KO primary astrocytes in mice that showed impairment in incorporation and distribution of fatty acid metabolites, AA, and palmitic acid (Gu et al. 2010). Astrocytic α-synuclein has been proved to be involved in the non-cell-autonomous killing of neurons as depicted in α-synuclein inducible transgenic mice (Emanuele and Chieregatti 2015). The PD-related A53T α-synuclein was selectively expressed by astrocytes, due to which A53T mutant mice developed rapidly progressive paralysis along with astrocytosis, microglial activation, and midbrain DA and spinal cord motor neuron degeneration. These results establish that α-synuclein-mediated cytotoxicity to astrocytes contributes to the neurodegeneration observed in PD.

3. The **LRRK2** gene encodes for LRRK2 protein that has dual kinase and GTPase activity. The most common genetic cause known for PD is the mutations in the LRRK2 gene, which results in autosomal dominant Parkinsonism and presents a phenotype similar to idiopathic PD (Zimprich et al. 2004). Polyclonal antibody to LRRK2 has shown to intensely label LBs in PD. LRRK2 is constitutively

expressed in neurons and glial cells of the human brain. Postmortem analysis of control, idiopathic, and G2019S (most common PD-linked variant) LRRK2 PD patients has shown no difference in the morphological localization of LRRK2 in control and PD individuals (Trinh et al. 2014). However, real-time RNA analysis has identified differential reduction of LRRK2 mRNA expression in PD cases compared to control, thus suggesting that dysregulated LRRK2 mRNA expression contributes to PD pathogenesis (Sharma et al. 2011).

The major physiological function of LRRK2 is in the autophagy/lysosomal pathway, as determined by using LRRK2 inhibitors. A specific inhibitor of the kinase activity of LRRK2 induced in human neuroglioma cells stimulated macroautophagy without any alteration in the translational targets of mTORC1, suggesting that LRRK2 regulates autophagic vesicle formation independent of canonical mTORC1 signaling. In the presence of autophagy, the LC3 protein is lipidated and transported to the membrane of autophagic vesicles. When LRRK2 kinase activity was inhibited, it has been shown to increase the lipidation of LC3 in primary mouse astrocytes (Manzoni et al. 2013). Furthermore, the presence of green fluorescent protein-tagged, PD-causing LRRK2 mutants (R1441C, Y1699C, and G2019S) in primary mouse astrocytes has been proven to lead to an increase in lysosome size, confirming the role of LRRK2 kinase activity in lysosomal autophagy in PD astrocytes (Henry et al. 2015). Given the association between LRRK2 kinase and autophagy, using LRRK2 inhibitors may have potential therapeutic use in PD.

4. **PARK (Parkin)**. Parkin is the protein encoded by gene PARK2 (Parkin RBR E3 ubiquitin-protein ligase) and is known to cause genetic recessive PD (Koyano and Matsuda 2015). Parkin mutations in humans produce Parkinsonism depicting dysfunctional protein degradation, elevated reactive species generation, and abnormal neurotransmitter release (Choi et al. 2013). The parkin protein, being a ubiquitin ligase, directly links ubiquitin-proteasome to the substrate, causing protein degradation (Kawajiri et al. 2011). Parkin is mainly involved in mitophagy in collaboration with another gene, PINK1 (Vives-Bauza et al. 2010). Under steady-state conditions, Parkin is in the inactive state and gets activated on sensing mitochondrial dysfunction. In such a scenario, Parkin is selectively recruited to depolarized mitochondria following uncoupling with an exogenous reagent such as CCCP and then stimulates the autophagic removal of damaged mitochondria (Narendra et al. 2008).

   Clinical studies on PD brains with PARK2 mutations have shown accumulated α-synuclein inclusions in astrocytes (Braak et al. 2007; Hayashi et al. 2000). One study on cultured midbrain glia from WT and PK-KO mice found that PK-KO glial cultures had fewer astrocytes, more microglia, reduced proliferation, and increased proapoptotic protein expression (Solano et al. 2008a). PARK2 mutations result in a loss of function, and Parkin has been shown to have a role in astrocyte proliferation, as evident from the reduced proliferation of PARK2-KO astrocytes. In the absence of PARK2, astrocytes show decreased GSH levels and thus reduced neurotrophic capacity. Furthermore, there is prolonged impairment in mitochondria in the case of PARK2-KO astrocytes

but not neurons. In control conditions, in response to unfolded protein response, there is an increase in Parkin levels in astrocytes, conveying that Parkin may exhibit cell type-specific functions (Ledesma et al. 2002). Additionally, Parkin is involved in the astrocyte inflammatory response through the activation of IL-1β with Parkin downregulation and TNF-α activation with upregulated Parkin levels (Khasnavis and Pahan 2014). The effect of upregulated PARK2 levels was checked in a MPTP mouse model of PD in protecting DA neurons using cinnamon. MPTP treatment in mouse increased iNOS levels and decreased Parkin/DJ-1 levels in the SN, which was counteracted using a cinnamon supplement, resulting in reduced iNOS expression and increased Parkin in the SN (Khasnavis and Pahan 2014). Therefore, the upregulation of PARK-2 in astrocytes may have therapeutic importance in PD.

5. The **PLA2G6** gene encodes for calcium-independent iPLA2β or VIA/iPLA2β, an enzyme that catalyzes the release of fatty acids from phospholipids (Kinghorna and Castillo-Quana 2016; Ramanadham et al. 2015). VIA iPLA2β also plays an important role in regulating cellular calcium homeostasis by directing a type of calcium influx, capacitative calcium entry (Strokin et al. 2012). Functionally active VIA/iPLA2β is necessary for activation of Orai1, the pore-forming subunit of the calcium release-activated calcium channels. Mutations in iPLA2 are a cause of infantile neuroaxonal dystrophy and neurodegeneration with brain iron accumulation and have lately been shown to cause PD (Mori et al. 2019). iPLA2β inhibition has been shown to inhibit the release of the fatty acid AA from phospholipids in astrocytes (Xu et al. 2003). Tang et al. (2010) reported heterozygous PLA2G6 p.P806R (c.2417C>G) mutation in exon 17 to be a possible PD-related mutation (Lin et al. 2018). Further, in primary astrocytes, PLA2G6 loss has been associated with reduced calcium response. LPS treatment of WT astrocytes resulted in increased calcium responses in terms of amplitude and duration, attributed to increased expression and activity of VIA/iPLA2 suggesting a link with TLR4 signaling and hence inflammation (Strokin et al. 2011). ATP-mediated calcium signaling is supposed to be vital for interaction among astrocytes (Arcuino et al. 2002), which might be impaired in patients with iPLA2 mutations.

   The pathogenesis of PLA2G6 gene mutation in PD could be attributed to the abnormal metabolism of AA which is associated with increased neuronal excitability and seizure propagation (Gaoa et al. 2016). Excess AA leads to sustained activation of NMDA receptors that results in activation of the synaptosomal release of glutamate, decreased glutamate uptake by astrocytes, and increased glutamate uptake into synaptic vesicles—all of which contribute to the increased glutamate levels in the synaptic cleft and consequent excitotoxicity (Aroniadou-Anderjaska et al. 2012). VIA/iPLA2β loss also results in acyl-chain shortening in phospholipids, affecting ER homeostasis and neurotransmission and promoting α-synuclein aggregation, which is one of the major constituents of LBs (Mori et al. 2019).

6. **PINK1** encodes for PINK1, a protein kinase involved in mitophagy (Koyano and Matsuda 2015). PINK1 expression in the brain increases as embryonic

development progresses and is vital for the development of astrocytes. PINK1 deficiency causes defects in GFAP expression during early brain development and leads to decrease in differentiation of NSCs into GFAP-positive astrocytes (astrogenesis) (Joe et al. 2018).

Most of the mutations in PINK1 that are PD-related are loss-of-function mutations (Kawajiri et al. 2011). PINK1 deficiency brings about the defective proliferative response of astrocytes to EGF and FBS due to mitochondrial dysfunction through increased p38 MAPK activation, decreased AKT activation, and decreased EGFR expression. Booth et al. (2017) showed that this defective proliferation of astrocytes in the brain is controlled by EGFR signaling via an AKT/p38-dependent pathway. In addition, PINK1 mutation brings about mitochondrial dysfunction bringing about a reduction in ATP production, which also contributes to the decreased proliferation of astrocytes, with grave repercussions for neuroprotection and general brain health (Booth et al. 2017). KO of PINK1 in mice resulted in a reduction in the number of astrocytes compared with WT mice (Choi et al. 2013), and PINK1-deficient astrocytic cultures from postnatal mouse showed reduced proliferation (Choi et al. 2013, 2016). Additionally, the mitochondrial condition of the cells was affected, resulting in reduced ATP production, which also contributed to their decreased proliferation. Sun et al. (2018) showed that the loss of PINK1 alters glial proliferation and brings about alterations in the glial inflammatory profile. Glial proliferation leads to hypersensitized astrocytes and microglia that have greater levels of basal and triggered inflammatory cytokine release, NO production, and NLRP3 inflammasome activation (Ge et al. 2020). PINK1 deficiency decreases the expression of various miRNAs like mir-326, mir-330, and mir-3099 that are necessary for NSC differentiation to astrocytes (Choi et al. 2016), while critical signaling molecules required for astrogenesis like SMAD1/5/8, STAT3, and HES1 are unaffected (Joe et al. 2018).

7. **GBA** is a lysosomal enzyme involved in the catalysis of glycolipids to ceramide and glucose (Ginns et al. 1985). It is synthesized in the ER and carried to the lysosomes via LAMP2. The GBA mutations are associated with alterations in lipid levels that may lead to lysosomal storage disease, which can induce synucleinopathies (Gatto et al. 2019). Currently, GBA variants are the most common genetic risk factor associated with Parkinsonism (Velayati et al. 2010). PD patients with GBA mutations are likely to have an early onset and greater cognitive decline. GBA activity is also significantly lower in the SN and anterior cingulate cortex of sporadic PD brains. The increased PD risk in GBA mutation carriers varies with race. The mechanism by which this mutation promotes PD is still unclear, but the main pathways implicated are the accumulation of α-synuclein, impaired lysosomal function, inhibition of autophagy, and the generation of ER stress (Do et al. 2019). GBA dysfunction can lead to lysosomal insufficiency, reducing α-synuclein degradation and promoting its aggregation, as lysosomes are the major compartments for protein degradation (Velayati et al. 2010). α-Synuclein is degraded via CMA, which requires binding to the lysosomal receptor, LAMP2a (Vogiatzi et al. 2008). However, the A53T and A30P

mutant variants of α-synuclein, as well as the posttranslationally dopamine-modified variants of α-synuclein, block this pathway.

In Parkinsonism associated with GBA mutations, the pathogenesis can be explained by both gain and loss of function. GBA degrades glucocerebroside to ceramide, and these changes in ceramide metabolism are associated with α-synuclein aggregation and, in turn, LB formation (Do et al. 2019). GCase activity on reduction results in increase in levels of glucosylceramide, affecting autophagy and promoting α-synuclein accumulation by stabilization of α-synuclein oligomeric forms that in turn inhibit GBA function, causing glucosylceramide (GlcCer) accumulation that further attenuates α-synuclein aggregation.

The KO of GBA in mice in neurons, astrocytes, and oligodendrocytes increased expression of cathepsin lysosomal proteases in both astrocytes and neurons (Vitner et al. 2010). Another study done on primary GBA-KO astrocytes has demonstrated a reduced number of LC3-positive puncta, indicating further deficits in the autophagy pathway (Osellame and Duchen 2013). Further, astrocytic mitochondria from GBA-KO mice exhibited decreased mitochondrial resting membrane potential and increased mitochondrial fragmentation (Osellame and Duchen 2013).

8. **ATP13A2** is a lysosomal protein type 5 ATPase implicated in the transport of cations and other substrates across membranes involving enzymes that mediate the coupling of active substrate transport with the hydrolysis of ATP (Veen et al. 2014). ATP13A2 plays a role in manganese and Zn metabolism, mitochondrial bioenergetics, autophagy-lysosomal pathway, and most importantly, in α-synuclein metabolism (Park et al. 2015).
In PD, ATP13A2 expression is reduced, resulting in impaired Zn buffering capacity that leads to cytosolic Zn concentration elevation. This in turn results in a decrease in DWm, an increase in ROS production, mitochondrial fragmentation, and ATP depletion and the impairment in activity of lysosomal hydrolase by disrupting the ionic balance in lysosomes (Park et al. 2014). Reduction of ATP13A2 expression inhibits externalization of α-synuclein in exosomes, which leads to accumulation of a-synuclein.

An ATP13A KO study in astrocytes has shown increased expression of proinflammatory cytokines and decreased expression of anti-inflammatory cytokines and NFs, such as GDNF, in comparison to WT astrocytes. Conditioned medium from ATP13A2-KO astrocytes was assessed to be less neurotrophic than medium from WT astrocytes and was not as much able to protect DA neurons from MPP+ toxicity. Moreover, it was also shown that MPP+ toxicity resulted in a reduction in expression of ATP13A2 in astrocytes, indicating that MPP+ toxicity followed the same pathway to induce degeneration (Qiao et al. 2016).

5 Deleterious Astrocytic Changes in PD

Figure 3 schematically represents the pathophysiology of astrocytes in PD.

1. **The role of astrocytes in inflammation**—One of the hallmarks of PD pathophysiology is neuroinflammation, which is involved in DA neuronal death. The major inflammatory players in the brain are activated microglia and reactive astrocytes (Heneka et al. 2005). Astrocytes turn reactive to counteract any pathological change in the brain, and this is characterized by hypertrophy and overexpression of the intermediate filament GFAP, generating glial scar composed of cytokines and chemokines (Sofroniew and Vinters 2010).
Postmortem analysis of SNpc regions of five PD individuals in comparison to control showed a comparative increase in the astrocytic level of inflammatory cytokines, including TNF, IL-1β, IL-2, IL-4, and IL-6 (Hunot et al. 1999; Mogi et al. 1994, 1996). In other studies, activated microglia, cytokine accumulation, and NF-κB pathway activation was found in the CSF and brains of PD patients (Hunot et al. 1999; McGeer et al. 1988) and in most experimental models of PD (Castaño et al. 1998; Członkowska et al. 1996; Gao et al. 2002). Astrocyte reactivity is reported in the brain of PD and MPTP intoxicated individuals and in animal models of the disease (Forno et al. 1992).

   Irrespective of the cause, both familial and idiopathic forms of PD exhibit LB formation, microglia activation, and nigral DA neuron loss as common features. α-Synuclein mutations resulting in protein aggregation also produce robust microglia activation, highlighting the involvement of inflammation in PD that is both astrocytic and microglia-mediated. Microglial activation by intranigral administration of prostaglandin J2 (endogenous reactive product of inflammation) can result in selective DA neuronal loss in the SN, formation of ubiquitin- and α-synuclein-immunoreactive aggregates, massive microglia and astrocyte activation, and locomotor deficits in treated mice (Pierre et al. 2009).

   These postmortem and animal studies thus suggest the role of glial involvement in PD pathophysiology, either at initiation or during progression (Halliday and Stevens 2011). Even if neuroinflammation does not precede DA neuron dysfunction, activation of microglia and astrocytes is promoted by dying DA neurons secreting (Aloisi 2001; Kim and de Vellis 2005).

   α-Synuclein is endogenously expressed at low levels in astrocytes. However, postmortem PD brains have shown α-synuclein-positive inclusions both in astrocytes and neurons, which corroborate several studies that report exchange of α-synuclein among neurons and astrocytes (Braak et al. 2007; Halliday and Stevens 2011; Lee et al. 2010c). In astrocytes, RNA analysis has identified the genes performing engulfment and phagocytosis, which highlights the vital role of glial dysregulation as a mechanism underlying PD pathogenesis (Chung et al. 2015). Furthermore, a neurotoxic A1 astrocyte population is seen in PD, suggesting that defects in neuronal debris clearance by astrocytes may contribute to enhanced protein accumulation, leading to proteinopathy as observed in PD (Liddelow et al. 2017). Non-fibrillized α-synuclein has been seen in protoplasmic astrocytes in early PD too (Sorrentino et al. 2019). Astrocytes are known to take up α-synuclein from the extracellular space through a TLR4-independent endocytosis pathway (Rannikko et al. 2015). The endocytosed α-synuclein is then localized to the lysosome leading to its degradation. Astrocytes can degrade fibrillar α-synuclein as well as human α-synuclein purified from LB, proposing

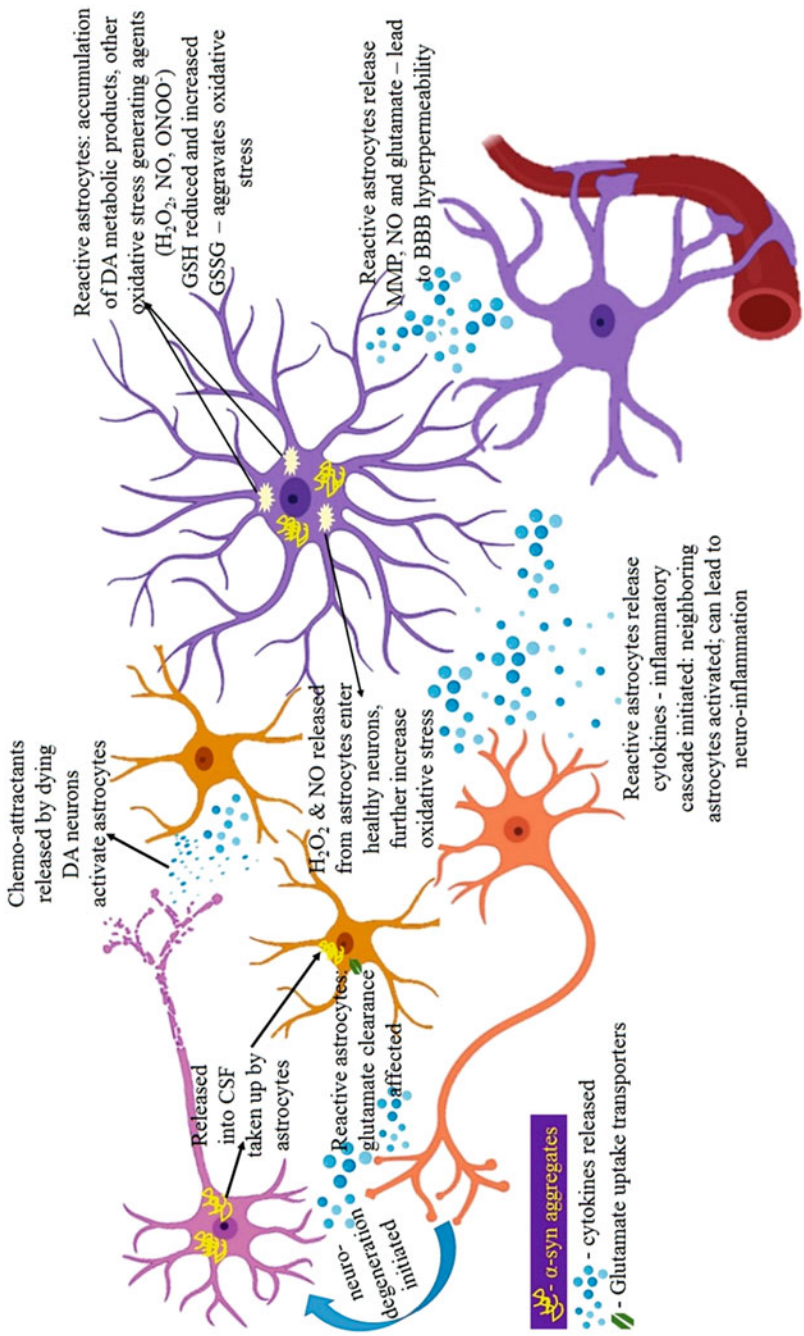

Fig. 3 Pathophysiology of astrocytes in PD: α-synuclein engulfed astrocytes can have deleterious effects in multiple ways. (Figure was prepared using BioRender (https://biorender.com/))

an active role of astrocytes in the clearance of α-synuclein deposits (Loria et al. 2017). However, in PD, the existence of α-synuclein with in the astrocytes has a negative impact on astrocyte functions. Above a certain threshold of α-synuclein, a TLR4-dependent inflammatory response was seen to be induced in primary astrocyte cultures (Lee et al. 2010a). On activation, astrocytes can indirectly activate microglia. This synergistic activation of astrocytes and microglia on α-synuclein induction was seen in animal models (Barcia et al. 2013) and can result in triggering the production of inflammatory factors such as TNF-α and IFN-γ in astrocytes (Lee et al. 2010a). The aggregation of α-synuclein in astrocytes breaks down their lysosomes and impacts mitochondrial homeostasis, leading to protein aggregation, and may induce cell-to-cell transfer of α-synuclein via tunneling nanotubes (Lindström et al. 2017). Thus, misfolded α-synuclein is not degraded in astrocytes and exchanged between different regions of the brain, leading to PD. In line with this, astrocytes from the PD cortex and striatum region have also shown to contain extensive α-synuclein deposits.

The cross talk between astrocytes and microglia in PD is further confirmed in a mouse model overexpressing A53T α-synuclein in astrocytes where α-synuclein aggregates led to astrogliosis and microglial activation—which resulted in the non-cell autonomous loss of DA and motor neurons and hence paralysis in the mouse (Gu et al. 2010). This study highlighted the pivotal role of astrocytic and microglial activation in causing neurodegeneration in the PD mouse model. Nevertheless, activated microglia have also been shown to induce reactivity of astrocytes, especially toward a pro-inflammatory, neurotoxic phenotype (Liddelow et al. 2017). Both astrocytosis and microglial activation, marked by IL-6 immunoreactivity, were also noticed in MPTP-administered mice, where a transient microglial reaction was observed on MPTP induction, followed by astrocytosis the next day, and continued until the end of the observation time (21 days) leading to depletion of DA cells (Kurkowska-Jastrzębska et al. 1999). Another study linking neuroinflammation to PD was conducted on mice overexpressing human α-synuclein. Increased expression of α-synuclein resulted in microgliosis and higher levels of inflammatory cytokines IL-1α, IL-6, TNF-α, COX-2, and iNOS, which preceded the loss of DA neurons (Theodore et al. 2008). This confirms that α-synuclein induction alone is quite sufficient to trigger a robust inflammatory cascade.

In silico analysis of a whole-genome transcriptome data set has predicted increased expression of chaperones belonging to the DnaJ/Hsp40 family in PD (Moran et al. 2006). Hsp belonging to molecular chaperones prevent the aggregation and promote ubiquitination as well as degradation of misfolded proteins (Chuang and Madura 2005). In PD astrocytes, the expression of DnaJB6, a novel component of LBs, was found to be highly expressed compared to its control counterparts. The upregulation of DnaJB6 in parkinsonian astrocytes further emphasizes the α-synuclein immunoreactivity in astrocytes (Durrenberger et al. 2009).

When microarray analysis was conducted on primary astrocytes treated with a conditioned medium of α-synuclein expressing SH-SY5Y neuronal cells, major

changes were observed in genes coding for proinflammatory cytokines and chemokines (Lee et al. 2010a). The drastic increase in expression of IL-1α, IL-1β, IL-6, IL-18, and CSF-1, CSF-2, and CSF-3 is due to a strong inflammatory response from astrocytes upon exposure to neuron-derived α-synuclein. Also, the expression of the anti-inflammatory molecule TGFβ3 decreased significantly (by 80% after 24 h). Along with cytokines, changes in chemokine expression in astrocytes were also observed, with CC-type (CCL-3, CCL-4, CCL-5, CCL-12, CCL-20), CXC-type (CXCL-1, CXC-2, CXC-5, CXC-10, CXC-11, CXC-12, CXC-16), and CX3C-type (CX3CL1) chemokines showing higher response to neuron-derived extracellular α-synuclein (Tremblay et al. 2019).

However, whether astrogliosis was triggered or not, inflammatory events seemed to affect astrocytes as evidenced by the increased levels of myeloperoxidase (Choi et al. 2005), a key oxidant-producing enzyme during inflammation; milk fat globule-EGF-8 (Kinugawa et al. 2013), a factor involved in phagocytic recognition; and HO-1 (Schipper et al. 2009), reported in astrocytes in the parkinsonian brain.

2. **The role of astrocytes in oxidative stress**—Oxidative stress is a major contributing factor in the pathogenesis of PD. The sensitivity of the PD brain to ROS is greater, owing to the high levels of polyunsaturated fatty acids and reduced levels of antioxidants in the affected area, the SNpc. Oxidative stress is accompanied by astrocytic proliferation in PD. Postmortem brain sections from PD patients have revealed a mitochondrial complex I inhibition in SN, which leads to decreased synthesis of ATP and excess ROS generation (Mizuno et al. 1990). Due to the presence of oxidative stress, the DNA is found to be severely oxidized in PD patients. Studies on autopsy samples of PD patients have shown a decrease in nucleolar integrity in DA neurons of the SN compared to control individuals. The nucleolus, the stress sensor of the cell (Choi et al. 2005), was found to be oxidatively damaged, as measured by expression of nucleophosmin in both the clinical PD samples and in MPTP-induced PD animal models (Rieker et al. 2011). Various neurotoxins used to model PD in animals have also reported oxidative stress generation. MPTP disrupts complex I of the mitochondrial respiratory chain thereby enhancing superoxide formation, which then initiates neuron loss. Similarly, 6-OHDA impairs DA neuronal function through free radical-mediated mechanisms. Products generated by RNS, such as ONOO- and NO, are commonly reported in the brains of MPTP-injected animals (Choi et al. 2005) and LBs of PD patients (Souza et al. 2000). NO, being a free radical, reacts with proteins and lipids causing their oxidation (Iravani et al. 2002).

Compared to astrocytes, neurons contribute to the majority of oxidative stress generation in PD due to the presence of dopamine metabolism and its auto-oxidation, low levels of antioxidants such as GSH and SOD, and mitochondrial complex I inhibition. While astrocytes are more resistant to ROS-induced damage, they generate oxidative stress in PD in multiple ways. Astrocytes metabolize dopamine enzymatically by MAOs into H_2O_2. Being pro-oxidant and highly permeable, H_2O_2 crosses into the neighboring DA cells and contributes to ROS

generation. As glia contain high levels of antioxidants GSH and GPx, they can easily transform H_2O_2 to water to protect their milieu.

There are two major sources of endogenous ROS generation in the brain—mitochondria, where the electron transport chain leads to the generation of ROS, and the NOX pathway, which catalyzes the transportation process of electrons from NADPH to O_2 that eventually converts to ROS. Astrocytic mitochondria distributed all over the astrocytic cell body and long processes play an important role in astrocytic redox regulation. Its role in neurological diseases was studied using an astrocytic mitochondrial Tfam conditionally KO model that resulted in increased neuronal death under conditions of ischemic stroke. Further neuronal defects and behavior impairment were observed in animals having an astrocyte-specific deletion of mitochondrial m-AAA protease. METH, a monoaminergic toxin that is associated with an increased risk of developing PD, decreases levels of dopamine and dopamine transporters and results in astrogliosis in vivo by disrupting mitochondrial function and thus causing oxidative stress in astrocytes (Granado et al. 2013). There are seven members in this NOX family, of which NOX2 and NOX4 are mainly expressed in the brain—NOX2 in microglia and NOX4 in astrocytes. The activity of astrocytic NOX4 is seen to be upregulated in neurodegenerative diseases where it induces oxidative stress and astrogliosis. Similarly, in an inflammatory model of PD induced with LPS, the expression of the NOX complex is increased leading to the pathogenesis of PD.

Astrocytic RNS is the major reactive product found in PD as a result of astrocyte-derived oxidative stress. Astrocytes are shown to express three main NOS isoforms expressed in the brain, calcium/calmodulin-dependent nNOS, eNOS, and calcium-independent iNOS (Gabbott and Bacon 1996; Galea et al. 1992). To check the effect of astrocytic RNS on the survival of neurons in co-culture, astrocytes were stimulated with cytokines to produce NO and its metabolite ONOO-, and it was found that both NO and ONOO- inhibit the activities of mitochondrial respiratory complexes II/III and IV of neighboring neurons, thereby inducing neuronal damage (Stewart et al. 2000). In addition, aggregated α-synuclein induced the production of ROS causing lipid peroxidation and cell death in neuron-astrocyte co-culture systems, which was inhibited by pre-incubation of co-culture with D-PUFAs, which confirms the role of lipid peroxidation in inducing neuronal death (Angelova et al. 2015). In PD patients and animal models, a PD associated protein, Parkin is S-nitrosylated. S-nitrosylation of parkin inhibits its ubiquitin E3 ligase activity, thus resulting in the aggregation of proteins in neurons as observed in PD (Chung et al. 2004).

As a cellular response to genotoxic stress, poly(ADP-ribosyl)ation, specifically PARP-1, PARP-2, and PARP-3, is known to get activated in astrocytes, which results in depletion of glycolysis, ATP levels, inflammation, and eventually widespread neuronal death. In PD, a significant increase of PARP-containing nuclei in the DA neurons has been observed in immunohistochemical studies. In astrocytes, glutamate transmission is firmly regulated by ATP-dependent astrocytic glutamate transporters alkylating agent MNNG, astrocytic PARP-1 was found to be upregulated, which leads to defects in glutamate uptake and hence

bioenergetic depletion and eventually to a significant reduction in cultured cortical astrocyte survival (Tang et al. 2010).

In astrocytes, IGF-1 signaling plays a role in protecting neurons from oxidative stress. The association between IGF-1 and PD has been documented in several studies such as (Gibb and Lees 1988) the presence of abundant IGF-1 receptors in the SN (Gibb and Lees 1988) improved survival of embryonic DA and SN neurons with IGF-1 treatment (Mashayekhi et al. 2010) and (Gibb and Lees 1988) protection of neuronal cells in vitro from dopamine-induced toxicity (Chung et al. 2005; Offen et al. 2001). These studies imply that astrocyte secreted IGF-1 regulates DA-induced toxicity by acting against ROS generation. Under neurodegenerative conditions, Nod-like receptors/inflammasomes get activated in astroglial cells. On activation of cultured cortical astrocytes by LPS or ethanol, mitochondrial ROS are generated that mediate NLRP3 inflammasome activation (Bernhard et al. 2016).

The major detoxifying enzyme GSH is of paramount importance in PD as the level of GSH depletion is found to be directly proportional to the severity of the disease. The loss of GSH content is the earliest known indicator of nigral degeneration (Jenner 1993; Jenner and Olanow 1996). Astrocytes produce and store the majority of GSH and protect neurons from oxidative stress by releasing GSH into the extracellular milieu. The antioxidant drugs used in treatment regimens for PD also act by increasing GSH synthesis in astrocytes (Finsterwald et al. 2015). The levels of the reduced form of GSH are significantly decreased whereas oxidized GSH levels are normal in the SN of PD patients (Riederer et al. 1989). Along with this, the levels and activities of GSH enzymes such as γ-glutamyl transpeptidase (Sian et al. 1994), GPx (Mythri et al. 2011), and glutathione-*S*-transferase (Reynolds et al. 2008) are significantly altered.

Nrf2 is another antioxidant pathway that was found to be impaired in a postmortem study of PD patients. While Nrf2 was seen to be localized to the nucleus in PD, the response may not be sufficient to protect neurons from degeneration (Ramsey et al. 2007). In a Parkinson's model, astrocytic activation of Nrf2 by tBHQ protected against MPP+ neurotoxicity. Interestingly, antioxidant peroxyredoxin-2 and peroxyredoxin-3 levels are increased in the PD patient brains as revealed by IHC (Martin et al. 2015; Qu et al. 2007). 6-OHDA induction, when given to DA neurons and a mouse model of PD, was found to be protective via mechanisms involving peroxiredoxin on DA neurons in culture. Peroxiredoxin overexpression resulted in significant in vitro and in vivo neuroprotection by exhibiting antiapoptotic effects in DA neurons through the suppression of ASK1-dependent activation of the c-Jun N-terminal kinase/c-Jun and p38 pro-death pathways and also preserved motor functions in an animal model (Qu et al. 2007).

3. **Astrocyte influence on BBB dysregulation**—The BBB is a major functional barrier in the brain and is composed of endothelial cells, pericytes, and astrocytes. During neurodegenerative conditions like PD, BBB is disrupted resulting in inflammation, oxidative stress, and neurotoxicity. This disruption of the function of BBB is also found to be mediated by astrocyte-derived vascular permeability

factors that upregulate BBB permeability, such as vascular endothelial growth factors, MMPs, NO, glutamate, and endothelin-1.

BBB is regulated by the basal lamina, made up of collagen, laminin and fibronectin, and tight junctions, composed of CLN, OCLN, and ZO (Luissint et al. 2012). The end feet of astrocytes wrap around cerebral microvessels and regulate BBB functions via astrocyte-derived factors. Astrocytic end feet express the potassium channel Kir4.1 and AQP4 that plays a role in maintaining the ion and water balance (Gleiser et al. 2016). Through inflammatory molecules, astrocytes regulate the expression of the cell adhesion molecules like VCAM-1 and ICAM-1, thereby affecting circulating leukocyte infiltration into the CNS. In brain disorders, astrocytes turn to the reactive state and release various extracellular signaling molecules such as VEGF, MMPs, NO, glutamate, and endothelin which induce endothelial cell apoptosis resulting in BBB disruption (Michinaga and Koyama 2019).

Clinical studies have demonstrated BBB impairment in PD patients (Kortekaas et al. 2005; Ohlin et al. 2011). According to the Braak staging framework for PD spread, all the regions disrupted in Braak stage 1 have axon terminals surrounding the BBB. However, BBB protects all other regions, whose axon terminals reside within the brain, from blood-borne substances. Thus, as depicted by positron-emission tomography imaging and CSF analysis, BBB hyperpermeability is evident in PD. The same has been shown by a postmortem study of PD individuals which confirmed compromised BBB integrity in the striatum by methods such as erythrocyte extravasation, perivascular hemosiderin, and leakage of various serum proteins outside UEA-staining vessel walls (Gray and Woulfe 2015; Kortekaas et al. 2005). Kortekaas et al. (2005) found an increase in the brain uptake of BBB impermeable drugs, benzerazide and [11C] verapamil in PD patients and animal models, indicating BBB disruption in PD. Moreover, PD patients administered levodopa presented a decrease in cerebral blood flow associated with increased BBB permeability as depicted by positron-emission tomography (Hirano et al. 2008).

Animal studies have similarly supported the presence of BBB dysfunction in the MPTP and 6-OHDA PD mouse models (Carvey et al. 2005; Chen et al. 2008). 6-OHDA treatment of mouse resulted in astrogliosis and disruption of tight junctions (Carvey et al. 2005), whereas MPTP injection impaired striatal BBB integrity by increasing angiogenesis and extravasation of serum albumin into the brain parenchyma (Gray and Woulfe 2015). The expression and role of the BBB efflux pump P-glycoprotein are found to be decreased in the PD midbrain as revealed by positron-emission tomography.

The BBB impairment observed in PD has been associated with astrocytes in many ways. BBB dysfunction in PD contributes to reactive gliosis, which leads to the production of VEGF via activation of astrocytes. High amounts of VEGF produced results in oedema and hyperpermeability and had deleterious effects on DA neurons (Yasuhara et al. 2005). Aggregated α-synuclein following its uptake in astrocytes disrupted astrocytic glutamate transporters along with the ability of astrocytes to regulate the BBB.

In PD, the expression of cytokines IL-6, IL-1B, and TNF-α was found to be increased with a concurrent decrease in BBB proteins ZO-1 and occludin in tight junctions (Wong et al. 2004). These studies highlight the involvement of reactive astrogliosis in BBB disruption.

4. **Glutamate transporters in dysfunction astrocytes and PD**—The predominant excitatory neurotransmitter in the human brain is glutamate which is regulated by the EAAT subtypes (Roberts et al. 2014). Astrocytes are the major source of EAAT1 and EAAT2 in the brain (Gargioli et al. 2018) where they regulate glutamate levels at the synapse to avoid neuronal excitotoxicity and hyper-excitability (Colangelo et al. 2014; Zhang et al. 2016a). Among the two subtypes, the EAAT2/GLT1 is broad distributed among the forebrain, cerebral cortex, and hippocampus. Extracellular glutamate accumulation is mainly due to the impairment of EAAT2 and is related to Huntington's disease, AD, PD, and AMS.
5. **AQP4 dysfunction in astrocytes and PD**—AQPs are a group of integral trans-membrane proteins involved in maintaining brain homeostasis via regulation of water permeability across BBB. AQPs are subdivided among various types based on distribution with AQP1 and AQP4 localized to choroid plexus and astrocytic foot processes, respectively. AQP4 channels cluster along the astrocyte end foot processes of the BBB neighboring onto blood vessels (Lan et al. 2016).

   AQP4 expressed in endothelial cells mediates the bidirectional interaction between astrocytes and BBB components (Lanciotti et al. 2013; Stavale et al. 2013). In mouse studies, the expression of AQP4 was found to be higher in SNpc than in the neocortex as evident from the IF labelling and electron microscope quantitative immuno-gold analysis (Hoddevik et al. 2017). Astrocytic AQP4 regulates various biological functions of the CNS, such as maintaining CNS water balance, spatial buffering of extracellular potassium, calcium signaling, neurotransmission (Stavale et al. 2013), learning and memory formation, synaptic plasticity, the interaction between astrocytes, BBB maintenance, and adult brain neurogenesis. Evidence suggests that AQP4 dysfunction results in the onset and progression of various pathophysiological disorders, such as PD, depression (Robinson and Jackson 2016), neuromyelitis optica, AD, ischemia, epilepsy, cerebral edema, and stroke. In PD patients, AQP4 mRNA was downregulated in comparison to age-matched healthy controls (Thumburu et al. 2014). A decrease in AQP4 expression reduced the differential degeneration of midbrain DA neurons in experimental PD. Similarly, studies with AQP4 downregulation have found DA neurons to be more susceptible to neurotoxicity through the modulation of astrocytic NFs.
6. **EAAT2-AQP4 interactions in astrocytes and PD**—In the mammalian CNS, AQP4 is co-localized with glutamate transporters EAAT1 and EAAT2, as well as with Kir 4.1 in the astrocyte plasma membrane, which suggests that AQP4 could modulate potassium ion and glutamate homeostasis (Wu et al. 2008). Immuno-histochemical analysis of healthy human subject's CNS tissue shows that there is co-localization of EAAT2 with AQP4 in gray matter astrocytes (Hinson et al. 2008). Several studies using co-immunoprecipitation, Western blot, and IHC have found region-specific co-localization between GLT1 and AQP4 in the

hippocampus, cerebellum, and cortex of AQP4 KO mice (Yang et al. 2013). In such mice, the glutamate levels were found to be elevated in the synaptic cleft, thereby inducing neurotoxicity in the hippocampus (Hubbard and Binder 2017). On the contrary, in an ENT1 null mice model, the colocalization of AQP4 with EAAT2 was found to be absent in striatal astrocytes (Mogoanta et al. 2014), but there was the interaction of AQP4 with GFAP in the striatum of these mice (Lee et al. 2013).

7. **Astrocyte dysfunction and iron homeostasis**—In PD, about 255% increase in intracellular iron was observed in the degenerating DA neurons and associated microglia in the SN in magnetic resonance imaging and ultrasound studies (Zhang et al. 2013). Further, neuromelanin granules associated with high amounts of iron are found in SN of PD patients (Kim et al. 2016); moreover, the ratio of Fe^{3+}:Fe^{2+} was significantly shifted from 2:1 in control to 1:2 in PD subjects. Animal studies in PD have demonstrated similar increases in iron levels in SNpc in different PD animal models, e.g., 6-OHDA and MPTP (Lee et al. 2012); lactacystin (Liddelow et al. 2017); and rotenone (Lindström et al. 2017) models. Being a highly reactive element, imbalance in iron homeostasis results in oxidative damage in the neurons. Free iron when present in higher amounts interacts with H_2O_2 and produces hydroxyl free radicals that are highly reactive. This chemical reaction is known as the Fenton Reaction and is the major cause of iron-induced neurodegeneration in neurons (Bishop et al. 2010). Iron-induced CNS neurotoxicity is associated with activation of the NMDA receptor signaling that involves NOS and proteins such as ferroportin and DMT1.

   Iron also plays a role as a cofactor for many key enzymes of neurotransmitter biosynthesis, such as dopamine and noradrenaline (Cherry et al. 2014). TH catalyzes the rate-limiting step for dopamine synthesis. In vitro studies have shown the regulation of TH activity by iron. Brain Fe homeostasis is maintained by an association of endothelial cells and astrocytes. Accumulated iron in SN, cerebellum, and basal ganglia increased iron-containing molecules, primarily ferritin, in astrocytes, implying that ferritin is the key iron storage protein in glial cells. There are two types of ferritin subunits, H-ferritin and L-ferritin, of which the H-subunit has ferroxidase activity for the changing iron from the ferrous to the ferric form (Berardelli et al. 2013). In PD substantia nigra, high amounts of H-ferritin but not L-ferritin have been observed. Also, PD patients and animal models of PD (induced by 6-OHDA, MPTP, and lactacystin) exhibited increased DMT1 levels in SN (Salazar et al. 2008).

   Iron stored as ferritin binds to the human TFR1 for its uptake. However, astrocytes lack TFR, which suggests that astrocytes take up iron through a different mechanism (Dexter et al. 1989). Endothelial cells expressing TFR1 take up Fe^{3+}-loaded Tf and reduce Fe^{3+} to ferrous Fe^{2+}. Ferrous iron is then transported from endosomes into the cytosol by DMT1 and exported into the extracellular fluid by ferroportin. Astrocytes then oxidize Fe^{2+} to Fe^{3+} using ceruloplasmin (Jiang et al. 2017). In PD, the buildup of high iron levels is found to be directly proportional to DA neuronal death and disease progression. This accumulated iron in PD leads to the formation of LBs composed of

aggregated α-synuclein. Iron, when injected into the midbrain, was found to increase levels of the "labile iron pool," thereby inducing an impairment in dopamine metabolism (Zamanian et al. 2012) and increased levels of lipid peroxide generation, leading to neuronal loss (Zecca et al. 2005).

High levels of metallothionein, and antioxidants such as GSH and manganese SOD, make astrocytes more resistant to iron-induced damage compared to neurons. Astrocytes expressing both TFR and ferritin tightly regulate iron overload in neurodegenerative conditions.

6 Neuroprotective Role of Astrocytes in PD

In addition to the predominant dysfunction of astrocytes observed in PD, astrocytes also affect neuroprotection in various ways, such as by releasing NFs like BDNF, GDNF, and MANF; by producing the antioxidant substrate GSH and enzymes such as GPx and SOD; and by activating the pathway (Nrf2 pathway).

1. **Involvement of NFs** (BDNF, GDNF, MANF)—Initial co-culture experiments showed that primary dopamine neurons survived better with the addition of conditioned media from astrocyte cultures (Lin et al. 1998), which led to the discovery of NFs. These NFs are diffusible factors produced by astrocytes and play a role in the development, maintenance, repair, and survival of neuronal populations (Knott et al. 2002). While the astrocyte-secreted NFs such as GDNF, BDNF, and MANF is considered neuroprotective, they have not been used directly in PD drug therapy because of their inability to cross the blood-brain barrier and their rapid degradation in vivo.
   GDNF plays a prominent role in improving the survival of VM DA neurons in culture and animal models of PD (Eggert et al. 1999). Transcriptomic analysis has shown the presence of GDNF in both the developing and adult rat midbrain and striatum (Choi-Lundberg and Bohn 1995). Among human fetuses at 12–15 weeks of gestation, mRNA expression of GDNF was detected in astrocyte cultures (Moretto et al. 1996), but it decreased in adulthood and was only seen in oligodendrocytes (Zhang et al. 2016b). A few studies have presented GDNF as a potent survival factor for VM DA neurons (Hegarty et al. 2014; Tomac et al. 1995), which led to the start of clinical trials of GDNF administration. In one study, clinical administration of GDNF to PD patients directly into the putamen resulted in significant improvement in patient's motor symptoms (Gill et al. 2003); however, other placebo-controlled trials produced no change in motor function (Lang et al. 2006a).

   In 6-OHDA treated mice, GDNF-producing astrocytes on pre-implantation into the midbrain prevented the acquisition of amphetamine-induced rotational behavior and completely prevented dopamine depletion within the SN. While GDNF was found to protect and repair DA neurons in 6-OHDA and MPTP models of PD in rodents and nonhuman primates, it failed to rescue DA neurons in an α-synuclein animal model of PD and did not result in any improvement in

behavior (Decressac et al. 2011). This is a hurdle in employing GDNF as a therapy, as α-synucleinopathy is the major hallmark of PD pathophysiology.

BDNF was the first protein identified that directly promotes the growth and survival of DA neurons in vivo. It has neurotrophic effects influencing a variety of non-cholinergic systems along with neurons, including dorsal root ganglion cells and hippocampal and cortical neurons. BDNF is involved in the maturation of astrocytes, with high expression of its receptor TrkB in cortical astrocytes. The neuroprotective effect of astrocytes on primary midbrain culture under 6-OHDA induction was seen to be mediated by BDNF secretion, which led to the differential effect of region-specific astrocytes on TH-positive neuron survival under 6-OHDA conditions. To check the effect of BDNF on damaged DA neurons, astrocytes transduced with the human BDNF gene were grafted into the striatum of the partially-lesioned hemiparkinsonian rat. BDNF astrocytes in such rats attenuated amphetamine-induced rotation by 45%, 32 days after grafting.

Transcriptome analysis of BDNF has shown a twofold higher presence of BDNF mRNA in astrocytes compared to neurons in the mouse cerebral cortex (Zhang et al. 2014). On the contrary, BDNF mRNA levels in human astrocytes and neurons are very low (Zhang et al. 2016a), which were seen to be increased through stimulation with KCl and glutamate in rat forebrain astrocytes in vitro (Wu et al. 2004).

MANF, compared to BDNF and GDNF, is a relatively new NF and is selective in promoting the survival of DA neurons of the ventral midbrain, apart from having a role in the neurite extension and neuronal differentiation and migration (Tseng et al. 2017). Unlike other factors, these proteins localize to the ER under normal conditions, where they inhibit cell proliferation and ER stress-induced cell death. In neurons, they are secreted after ER calcium depletion and in response to ER stress (Lindahl et al. 2017).

In the developing mouse brain, high levels of MANF mRNA were detected in astrocytes compared to neurons (Zhang et al. 2014). The highest levels detected in mouse astrocytes remain relatively stable throughout the lifespan of the animal, up to 2 years of age (Clarke et al. 2018). Experimental studies on 6-OHDA induced PD in rats have shown the neuroprotective role of intrastriatal injected MANF as evaluated by TH staining in SN and striatum. Also, pre-administration of MANF in the striatum restored the function of the nigrostriatal DA neurons in 6-OHDA induced PD model in rats. In vitro studies using SH-SY5Y have shown the protective action of MANF against 6-OHDA toxicity by inhibiting the ER stress and activating the PI3K/Akt/mTOR pathway (Thorburne and Juurlink 1996). As ER stress is involved in PD pathogenesis, MANF may have a neuroprotective role in the disease in addition to playing the role of a trophic factor.

In sum, astrocytes secrete NFs BDNF, GDNF, and MANF that have a neuroprotective action on DA neurons in a PD model (Chen et al. 2006).

2. **Antioxidant defense of astrocytes**—GSH, GPx, SOD—compared to other brain cells, astrocytes are more resistant to oxidative stress, which could be due to the higher amounts of GSH present in astrocytes than in neurons in the adult brain.

Higher cytosolic GSH concentration and GPx activity were detected in astrocytes than neurons (Huang and Philbert 1995). At the same time, the pro-oxidant iron content was also found to be 20-fold less in astrocytes compared to neurons. Dopamine being a reactive molecule can oxidize to form O^{2-}, H_2O_2, and reactive quinones. The metabolism of dopamine thus generates a redox state, resulting in the generation of ROS (Zeevalk et al. 2008).

GSH—The concentration of GSH in astrocytes (~3.8 mmol/L) is estimated to be higher than that in neurons (~2.5 mmol/L) (Bolanos et al. 1995; Rice and Russo-Menna 1998), due to higher specific activity of the γ-glutamylcysteine synthetase in astrocyte (Gegg et al. 2003). Due to their proximity, astrocytes can share their GSH with neighboring neurons by releasing this antioxidant substrate through the MRP1 transporter into the extracellular space (Hirrlinger et al. 2002b). The GSH content in the SN of PD patients is significantly reduced (~40%) (Sian et al. 1994), and at a cellular level, significant loss of GSH is reported in surviving nigral DA neurons (Pearce et al. 1997). In genetic models of PD, culture aged astrocytes from PK-KO mice show lower levels of GSH than those from WT animals (Solano et al. 2008a). The clinical trial of parenteral GSH administration in PD patients failed to show any clinical benefits (Hauser et al. 2009), most likely due to the low permeability of GSH across the BBB (Cornford et al. 1978; McLellan et al. 1995).

GPx is one of the antioxidant enzymes that catalyze the reduction of H_2O_2 to water, thereby maintaining intracellular homeostasis (Damier et al. 1993). According to IHC data, the expression of GPx in normal and PD patient brains was exclusively confined to glial cells and was not detectable in neurons. In PD patient brains, the GPx immunoreactive astrocytes were observed to be surrounding the surviving DA neurons, thereby demonstrating their neuroprotective role (Power and Blumbergs 2009). To study the effect of GPx1 in H_2O_2 detoxification, a GPx1-deficient mouse line was developed, which revealed that astrocytes from GPx1 deficient mice are more vulnerable to H_2O_2-induced ROS generation, as they dispose of exogenous H_2O_2 more slowly than wild-type cells. When compared to WT astrocytes, GPx1-deficient cells did not accumulate oxidized glutathione after peroxide application (Liddell et al. 2006; Lubos et al. 2011; Power and Blumbergs 2009).

The antioxidant role of GPx in GCM in thrombin-treated human astrocyte cultures was evaluated to show the neuroprotective effect of the conditioned medium in thrombin-treated human astrocytes. The inclusion of a GPx inhibitor in GCM-Thr abolished its neuroprotective function, implying that the protection is mediated mainly by the increased amount of GPx released in the astrocyte-conditioned medium.

Further, the potency of GPx1 on DA neurons of 6-OHDA induced PD was studied using SK-N-MC cells (human neuroblastoma cell line) transduced with a human GPx1 lentiviral construct. This led to increased GPx1 protein expression and enzymatic activity, resulting in inhibition of the increase in intracellular ROS generation. In addition of astrocyte conditioned media, H_2O_2 production was significantly reduced in both GPx1 and non-transduced cells (Lei et al. 2007).

However, because of GPx1 overexpression, the addition of astrocyte-CM before 6-OHDA treatment could suppress H_2O_2 to levels nearly close to that in healthy counterpart cells (Bensadoun et al. 1998).

SOD—The role of oxidative stress and that of antioxidant SOD were studied in a drosophila model of PD created by the overexpression of α-synuclein. In this in vivo model, α-synuclein cytotoxicity resulted in both DA cell loss and locomotor disabilities shown in flies (Botella et al. 2008). Overexpression of human Cu/Zn SOD was sufficient to protect DA neurons against the toxic effect of mutant α-synuclein, suggesting that oxidative stress in DA neurons plays an important role in α-synuclein toxicity (Thiruchelvam et al. 2005). In another in vitro study, SOD could protect against dopamine toxicity in cultured neuroblastoma cells. Clinical studies in PD patients have shown an increase in SOD levels in the SN and motor cortex of the PD brain, indicating the importance of SODs in PD development (Çokal et al. 2017).

Nrf2 Pathway—In PD, increased oxidative stress is correlated with neuronal cell death (Navarro and Boveris 2009). The major regulator of the cytoprotective response to oxidative stress is the Nrf2-ARE pathway. In basal conditions, the transcription factor Nrf2 is bound to the Keap1 element in the cytoplasm. Under stress, Nrf2 proteins get dissociated from Keap1 and translocate into the nucleus, where they bind to the ARE and drive the expression of downstream antioxidant and detoxification genes such as NQO1, HO-1, glutamate-cysteine ligase, and GSTs (Lee and Wei 2005).

Studies have indicated that astrocytic Nrf2 expression mediates neuroprotection in the MPTP model of PD (Chen et al. 2009). Postmortem analysis of PD patients has shown Nrf2 localization and expression in the nucleus, which may be insufficient to protect neurons from degeneration (Ramsey et al. 2007). Nrf2 protein levels isolated from the CSF of PD patients with LRRK2 mutation (G2019S) were found to be positively correlated with disease duration, motor scores, and the Unified PD Rating Scale 43. Unlike the cytosolic expression of Nrf2 in healthy patients, it localizes to the nucleus of nigral DA neurons in early Braak stage (1–2) PD patients. This translocation of Nrf2 from the cytoplasm to the nucleus in PD patients indicates the response to increased ROS. The Nrf2-downstream gene NQO1 metabolizes dopamine-derived quinones and is seen to be expressed at higher levels within the SNpc of PD patients compared to healthy controls (Paladino et al. 2018; van Muiswinkel et al. 2004). In addition, overexpressed NQO1 protects cells against dopamine-mediated mitochondrial damage in vitro and reduces MPTP toxicity in vivo (Jazwa et al. 2011). Another Nrf2-transcribed antioxidant HO-1 is also expressed at higher levels in PD patient blood sera in comparison to healthy control (Flier et al. 2002).

7 Cell-Based Treatment Strategy to Target Astrocyte Regeneration and Replacement

Astroglia transplantation is considered a targeted therapy due to the survival of engrafted astroglial cells in pathological conditions. Astrocytes can be generated from different types of cells such as ESCs, iPSCs, MSCs, and DPSCs.

1. **ESCs** represent an ideal pluripotent source for deriving human astrocytes (Fig. 4). Astrocytes have been generated from ESCs by various groups (Krencik et al. 2011). Krencik et al. (2011) have come up with a robust protocol for generating immature astrocytes with high efficiency from hPSCs. The protocol involves three steps—the differentiation of hESCs to neuroepithelial cells (days 0–21), generation of astroglial subtypes by regular dissociation of the neuroepithelial clusters in suspension (days 21–90), and amplification of astroglial progenitors or differentiation into functional astrocytes (days >90). The advantages of this protocol include the lack of immune cells such as microglia, and the generation of astrocytes with specific regional and functional characteristics (Krencik et al. 2011). Similarly, Izrael et al. (2007) have developed an original and promising cell therapy approach to ALS using hES-AS. While ESCs serve as the best candidates for the generation of functional astrocytes, certain serious shortcomings such as ethical constraints, potential teratogenicity of these cells, time-consuming protocols (>180 days in culture), and the difficultly of scaling up have shifted the focus to adult stem cells such as MSCs.
2. **iPSCs** are obtained by genetic reprogramming of adult cells and have characteristics close to ESCs. iPSCs can be an effective alternative for ESCs providing an inexhaustible supply of astrocytes. Astrocytes have been successfully generated by different groups from iPSCs (Perriot et al. 2018; Tyzack et al. 2016). Perriot et al. (2018) developed a serum-free, high output, shorter-duration protocol to differentiate hiPSCs into astrocytes. The protocol involves inducing the neutralization of hiPSCs into NSCs using dual SMAD signaling inhibition (SB431542 with Noggin), followed by the treatment of cells with leukemia inhibitory factor (Iliff and Nedergaard 2013), and EGF to switch towards glial cell differentiation by activation of the JAK-STAT pathway. This protocol results in a high proliferative population of astrocyte-committed GPCs in 14 days yielding potentially more than one billion GPCs from two to three million hiPSCs. Moreover, there are commercially available kits used for deriving astrocytes from iPSCs, like the Axol's Human iPSC-derived Astrocyte Kits that include cryopreserved cells, optimized media, and supplements.
3. **MSC**-derived astrocytes secrete various NFs and cytokines, have immunomodulatory, anti-apoptotic, and anti-inflammatory effects and modulate reactivity/phenotype of astrocytes and the microglia, thereby promoting neuroregeneration, making them an ideal candidate for astrocyte derivation (Fig. 5). Stroomza et al. (2009) successfully differentiated BM-MSCs to astrocytes and successfully showed their impact on a PD model. Quantitative analysis of these astrocyte-like cells yielded significant amounts of GDNF, NGF, and BDNF as measured by real-time polymerase chain reaction, ELISA, and western blot analyses. In vivo

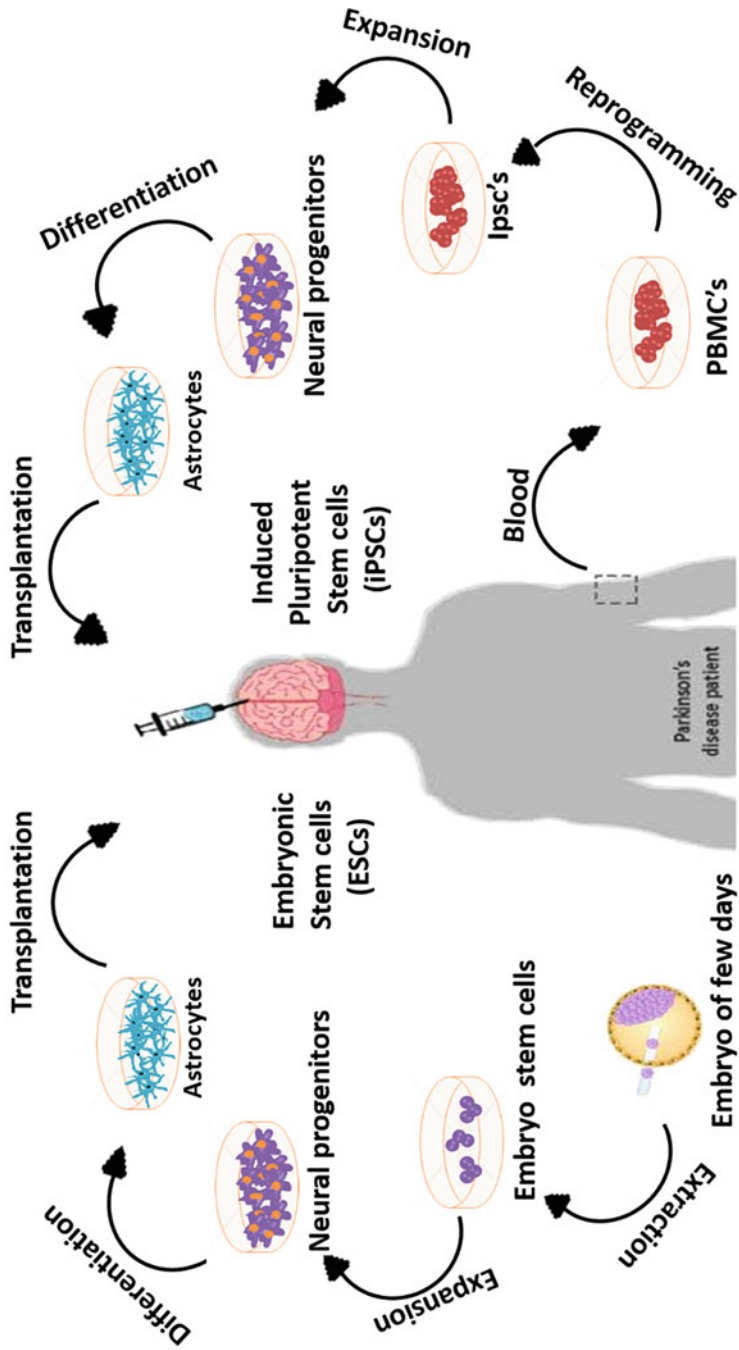

Fig. 4 Pluripotent stem cells such as ESCs and iPSCs serve as the best candidates for the generation of functional astrocytes. (Figure was prepared using BioRender (https://biorender.com/))

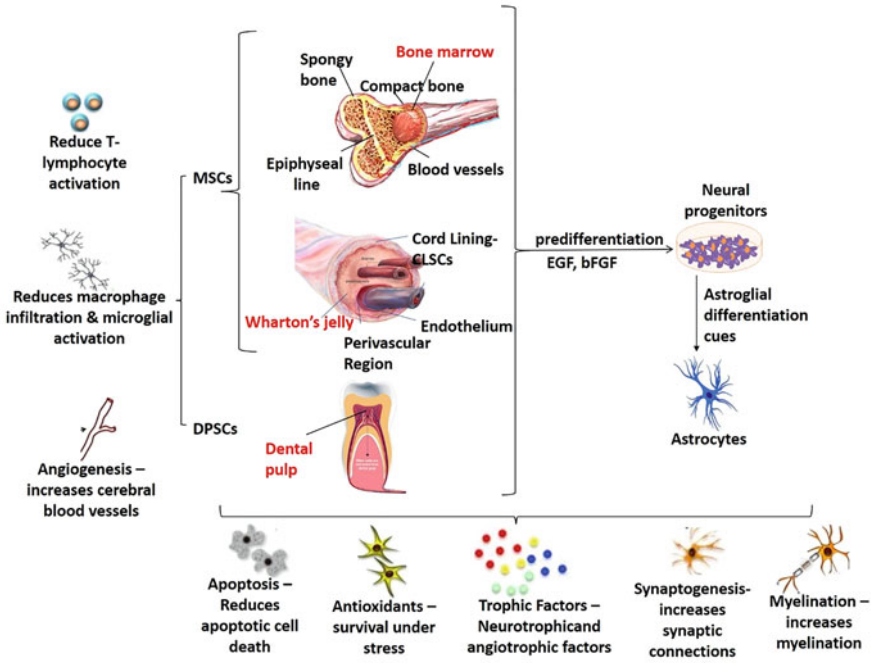

Fig. 5 MSCs and DPSCs in their naïve and differentiated astrocyte form have the capacity of promoting neuro-regeneration. (Figure was prepared using BioRender (https://biorender.com/)

studies with transplantation of BM-MSC-derived astrocytes into the striatum of 6-OHDA-lesioned rats in a PD model showed a reduction in the apomorphine-induced contralateral rotations, as well as behavioral improvement. Histological analysis revealed that the engrafted astrocytes survived, expressed astrocytes and human markers, and helped to regenerate the damaged DA nerve terminal system. Thus, suggesting that MSCs can render neuroprotection to DA neurons under stress.

4. **DPSCs** are another potential source of cells used for neurodegenerative diseases because they originate from neural crest cells (Janebodin et al. 2011) and share a common origin with peripheral nerve glial progenitor cells (Kaukua et al. 2014). There are minimal ethical concerns involved with dental pulp-derived MSCs compared to MSCs collected from bone marrow or adipose tissue (Caseiro et al. 2016; Seo et al. 2004). It is possible for DPSCs to differentiate into neuron-like cells and secrete NFs such as NT, BDNF, NGF, VEGF, and GDNF at remarkably higher levels compared to MSCs derived from bone marrow and adipose tissue (Mead et al. 2014), which play pivotal roles in neuroprotection and neuritogenesis (Fig. 5) (Xiao and Le 2016). DPSCs also express receptor EP2 at a higher level compared to both BM-MSCs and AMSCs, which has an important role in the release and synthesis of NTs (Duarte et al. 2012). There is compelling evidence that compared to other MSCs, DPSCs display higher neuroprotective

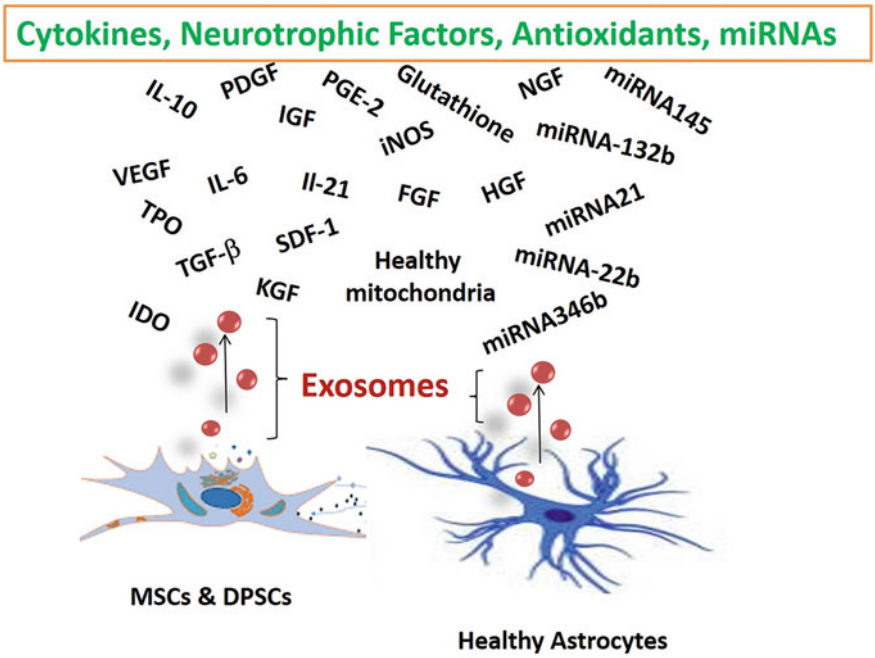

Fig. 6 Exosomes of MSCs and healthy astrocytes carry a cargo of multiple factors which has the potential for neuro-repair. (Figure was prepared using BioRender (https://biorender.com/))

and neurotrophic properties in reaction to injuries and pathologies of the nervous system (Li et al. 2017). DPSCs are non-tumorigenic and share similar immunomodulatory properties, mesenchymal surface marker profile, and trilineage differentiation potential with BM-MSCs. There are fewer ethical concerns for tissue extraction, higher proliferation rate, and single CFU-F than BM-MSCs, making it an attractive alternative source for astrocyte differentiation (Heng et al. 2016). In vitro induction of DPSCs to astrocytes with differentiation, factors showed a definitive increase in astrocyte-specific markers GFAP and EAAT2 along with glial calcium-binding protein S100β through flow cytometry and IF assays (Ganapathy et al. 2019). A significant increase in BDNF and GDNF expression and secretion in astrocyte-differentiated DPSCs was reported over naïve DPSCs and these cells were capable of protecting DA neurons from 6-OHDA stress.

5. **Exosomes derived from healthy astrocytes, MSCs and DPSCs**. Exosomes are naturally occurring nano-sized vesicles deliminated by a phospholipid bilayer, typically ranging between 30 and 100 nm in size, which is released from the endocytic compartment of live cells. They are packed with necessary neurotrophic factors secreted by their parent-cells and can also be ideal delivery vehicles as they are proven to cross BBB, unlike cells from which they originate (Fig. 6) (Perets et al. 2018; Wu et al. 2017). These nanoscale lipid bilayer exosomes carry lipids, mRNA, miRNA, and proteins derived from the parental

cell and transfer them to recipient cells to exert function (Théry et al. 2002). Exosomes can overcome key limitations of cell therapy with the potential advantages of being stable, biocompatible, having reduced clearance rate, minimal long-term accumulation in any organ or tissue with concomitant low systemic toxicity, and with the ability to cross the blood-brain barrier easily (van der Pol et al. 2012). They also have the possibility of delivery through the intranasal route due to their smaller size and good immune tolerance (Aminzadeh et al. 2017; De et al. 2017).

It is possible to use exosomes derived from astrocytes as an alternative to astrocyte cell therapy to induce transcriptomic and phenotypic changes required for neuroregeneration (Ibrahim and Marbán 2016). Exosome miRNA content is specific for the parental cell and the cell condition (e.g., hypoxia, inflammation) (Agnieszka et al. 2016). While blood-delivered exosomes usually accumulate in the liver, kidney, and spleen, they may have targeted specific organs through the presence of different cell-binding receptor proteins or integrins on their surface (Liu and Su 2019). Previous studies have shown that exosomes from cultured astrocytes could contact co-cultured neurons to promote neurite outgrowth of neighboring neurons and/or neuronal survival (Hira et al. 2018; Janas et al. 2016; Pei et al. 2019; Xu et al. 2019).

Increasingly exosomes from MSCs and DPSCs are gaining prominence in cell-based therapy because they exhibit low-immunogenicity, higher-biocompatibility, and minimum-toxicity. In addition, DPSC-exosomes display similar neuromodulatory and neuroprotective mechanisms as DPSCs themselves (Jarmalavičiūtė et al. 2015). Further, unlike nanoparticles, exosomes can avoid the MPS to deliver cargo and do not get destroyed (Hall et al. 2016). Two groups have explored the effect of exosomes on the Parkinson's disease model. Elena-Batrakova's group (Haney et al. 2015; Zhao et al. 2014) have used exosomes derived from bone-marrow BM-MSCs and genetically modified macrophages for delivery of catalase and GDNF, respectively, in PD-model, while Kojima et al. (2018) have reported the intracerebral delivery of designer exosomes produced by implanted cells as therapeutic cargo for PD treatment (Kojima et al. 2018). The beneficial effects of exosomes have been reported for behavioral motor function parameters and IHC data has been used to detect the presence of exosomes in the midbrain of animal model. If cell-based therapeutic effects can be mimicked by the exosomes, then it is a boon because the use of MSC-exosomes can avoid the challenges of MSC transplantation like maintenance of biological activity, quantification of bioactive substances, and logistics of delivery of MSCs in clinical therapies. Moreover, a cell-free exosomal system for delivery of miRNA or antioxidants can act as a feasible therapeutic means for reaching the midbrain through the intranasal route.

8 Conclusions

Glial cells were initially assumed to serve as support tissue in the brain, with no active contribution to information processing in the CNS. However, brain research over the past few decades has established the active role of glial cells in neurodegenerative diseases such as PD. Issues ranging from the presence of α-synuclein immunoreactive inclusions in astrocytes of PD patient brains, to the expression of genes with causative relevance to PD in astrocytes and the eventual effect on astrocyte function, have been discussed in detail in this chapter. Astrocytes are multifunctional and form the appropriate niche for DA neurons, contributing to the survival and maintenance of neuronal health, ion buffering, neurotransmitter recycling, and regulation of the blood-brain barrier. Further, the complex role of astrocytes is influenced by region specificity and the number of astrocytes, which affect the survival of DA neurons. Understanding these niche cells is also of prime importance for designing approaches for prophylactic and regenerative strategies through derivation of exogenous niche cells from patient-specific iPSCs or mesenchymal stromal cells. Here, we have reviewed the potential protective and deleterious effects of astrocytes in the SNpc of PD and explored how recent developments can in turn impact our understanding of the pathophysiology of PD and its treatment.

References

Agnieszka B, Jakub G, Antonio Chiocca E (2016) Extracellular vesicles and microRNAs: their role in tumorigenicity and therapy for brain tumors. Cell Mol Neurobiol 36(3):361–376

Aloisi F (2001) Immune function of microglia. Glia 36(2):165–179

Aminzadeh M, Rogers R, Gouin K (2017) Reversal of cardiac and skeletal manifestations of Duchenne muscular dystrophy by cardiosphere-derived cells and their exosomes in mdx dystrophic mice and in human Duchenne cardiomyocytes. bioRxiv

Angeles DC, Gan BH, Onstead L, Zhao Y, Lim KL, Dachsel J, Melrose H, Farrer M, Wszolek ZK, Dickson DW (2011) Mutations in LRRK2 increase phosphorylation of peroxiredoxin 3 exacerbating oxidative stress-induced neuronal death. Hum Mutat 32(12):1390–1397

Angelova PR, Horrocks MH, Klenerman D, Gandhi S, Abramov AY, Shchepinov MS (2015) Lipid peroxidation is essential for α-synuclein-induced cell death. J Neurochem 133(4):582–589

Arcuino G, Lin JH-C, Takano T, Liu C, Jiang L, Gao Q, Kang J, Nedergaard M (2002) Intercellular calcium signaling mediated by point-source burst release of ATP. Proc Natl Acad Sci U S A 99(15):9840–9845

Argaw AT, Asp L, Zhang J, Navrazhina K, Pham T, Mariani JN, Mahase S, Dutta DJ, Seto J, Kramer EG, Ferrara N, Sofroniew MV, John GR (2012) Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. J Clin Invest 122(7):2454–2468

Aroniadou-Anderjaska V, Pidoplichko VI, Figueiredo HT, Almeida-Suhett CP (2012) Presynaptic facilitation of glutamate release in the basolateral amygdala: a mechanism for the anxiogenic and seizurogenic function of GLUK1 receptors. Neuroscience 221:157–169

Auluck PK, Caraveo G, Lindquist S (2010) α-Synuclein: membrane interactions and toxicity in Parkinson's disease. Annu Rev Cell Dev Biol 26:211–233

Bandopadhyay R, Kingsbury AE, Cookson MR, Reid AR, Evans IM, Hope AD, Pittman AM, Lashley T, Canet-Aviles R, Miller DW (2004) The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. Brain 127(2):420–430

Barcia C, Ros CM, Ros-Bernal F, Gómez A, Annese V, Carrillo-de Sauvage MA, Yuste JE, Campuzano CM, De Pablos V, Fernández-Villalba E (2013) Persistent phagocytic characteristics of microglia in the substantia nigra of long-term Parkinsonian macaques. J Neuroimmunol 261(1–2):60–66

Barres BA (2008) The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60(3):430–440

Bayraktar OA, Fuentealba LC, Alvarez-Buylla A, Rowitch DH (2015) Astrocyte development and heterogeneity. Cold Spring Harb Perspect Biol 7(1):a020362

Bensadoun J, Mirochnitchenko O, Inouye M, Aebischer P, Zurn A (1998) Attenuation of 6-OHDA-induced neurotoxicity in glutathione peroxidase transgenic mice. Eur J Neurosci 10(10): 3231–3236

Berardelli A, Wenning G, Antonini A, Berg D, Bloem B, Bonifati V, Brooks D, Burn D, Colosimo C, Fanciulli A (2013) EFNS/MDS-ES recommendations for the diagnosis of Parkinson's disease. Eur J Neurol 20(1):16–34

Bernhard FP, Heinzel S, Binder G, Weber K, Apel A, Roeben B, Deuschle C, Maechtel M, Heger T, Nussbaum S, Gasser T, Maetzler W, Berg D (2016) Insulin-like growth factor 1 (IGF-1) in Parkinson's disease: potential as trait-, progression- and prediction marker and confounding factors. PLoS One 11(3):e0150552

Bishop GM, Scheiber IF, Dringen R, Robinson SR (2010) Synergistic accumulation of iron and zinc by cultured astrocytes. J Neural Transm 117(7):809–817

Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ (2018) The aging astrocyte transcriptome from multiple regions of the mouse brain. Cell Rep 22(1):269–285

Bolanos JP, Heales SJ, Land JM, Clark JB (1995) Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurones and astrocytes in primary culture. J Neurochem 64(5):1965–1972

Bonifati V, Rizzu P, Van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299(5604):256–259

Booth HD, Hirst WD, Wade-Martins R (2017) The role of astrocyte dysfunction in Parkinson's disease pathogenesis. Trends Neurosci 40(6):358–370

Botella JA, Bayersdorfer F, Schneuwly S (2008) Superoxide dismutase overexpression protects dopaminergic neurons in a Drosophila model of Parkinson's disease. Neurobiol Dis 30(1): 65–73

Braak H, Sastre M, Del Tredici K (2007) Development of α-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. Acta Neuropathol 114(3):231–241

Braidy N, Gai W-P, Xu YH, Sachdev P, Guillemin GJ, Jiang X-M, Ballard JWO, Horan MP, Fang ZM, Chong BH, Chan DKY (2013) Uptake and mitochondrial dysfunction of alpha-synuclein in human astrocytes, cortical neurons and fibroblasts. Transl Neurodegener 2(1):20–20

Brichta L, Greengard P (2014) Molecular determinants of selective dopaminergic vulnerability in Parkinson's disease: an update. Front Neuroanat 8:152

Brown AM (2004) Brain glycogen re-awakened. J Neurochem 89(3):537–552

Cabezas R, Avila M, Gonzalez J, El-Bachá RS, Báez E, García-Segura LM, Jurado Coronel JC, Capani F, Cardona-Gomez GP, Barreto GE (2014) Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. Front Cell Neurosci 8:211–211

Carvey PM, Zhao CH, Hendey B, Lum H, Trachtenberg J, Desai BS, Snyder J, Zhu YG, Ling ZD (2005) 6-Hydroxydopamine-induced alterations in blood-brain barrier permeability. Eur J Neurosci 22(5):1158–1168

Caseiro AR, Pereira T, Ivanova G, Luís AL (2016) Neuromuscular regeneration: perspective on the application of mesenchymal stem cells and their secretion products. Stem Cells Int 2016: 9756973

Castaño A, Herrera AJ, Cano J, Machado A (1998) Lipopolysaccharide intranigral injection induces inflammatory reaction and damage in nigrostriatal dopaminergic system. J Neurochem 70(4): 1584–1592

Chaboub LS, Deneen B (2012) Developmental origins of astrocyte heterogeneity: the final frontier of CNS development. Dev Neurosci 34(5):379–388

Chen PS, Peng G, Li G, Yang S, Wu X, Wang C, Wilson B, Lu R-B, Gean P-W, Chuang D (2006) Valproate protects dopaminergic neurons in midbrain neuron/glia cultures by stimulating the release of neurotrophic factors from astrocytes. Mol Psychiatry 11(12):1116–1125

Chen X, Lan X, Roche I, Liu R, Geiger JD (2008) Caffeine protects against MPTP-induced blood-brain barrier dysfunction in mouse striatum. J Neurochem 107(4):1147–1157

Chen P-C, Vargas MR, Pani AK, Smeyne RJ, Johnson DA, Kan YW, Johnson JA (2009) Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: critical role for the astrocyte. Proc Natl Acad Sci U S A 106(8):2933–2938

Cherry JD, Olschowka JA, O'Banion MK (2014) Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. J Neuroinflammation 11(1):98

Chever O, Pannasch U, Ezan P, Rouach N (2014) Astroglial connexin 43 sustains glutamatergic synaptic efficacy. Philos Trans R Soc Lond Ser B Biol Sci 369(1654):20130596

Choi D-K, Pennathur S, Perier C, Tieu K, Teismann P, Wu D-C, Jackson-Lewis V, Vila M, Vonsattel J-P, Heinecke JW (2005) Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice. J Neurosci 25(28):6594–6600

Choi I, Kim J, Jeong H-K, Kim B, Jou I, Park SM, Chen L, Kang U-J, Zhuang X, Joe E-h (2013) Pink1 deficiency attenuates astrocyte proliferation through mitochondrial dysfunction, reduced akt and increased p38 mapk activation, and downregulation of egfr. Glia 61(5):800–812

Choi I, Woo J, Jou I, Joe E (2016) PINK1 deficiency decreases expression levels of mir-326, mir-330, and mir-3099 during brain development and neural stem cell differentiation. Exp Neurobiol 25:14–23

Choi DJ, Eun JH, Kim BG, Jou I, Park SM, Joe EH (2018) A Parkinson's disease gene, DJ-1, repairs brain injury through Sox9 stabilization and astrogliosis. Glia 66(2):445–458

Choi-Lundberg DL, Bohn MC (1995) Ontogeny and distribution of glial cell line-derived neurotrophic factor (GDNF) mRNA in rat. Brain Res Dev Brain Res 85(1):80–88

Chuang S-M, Madura K (2005) Saccharomyces cerevisiae Ub-conjugating enzyme Ubc4 binds the proteasome in the presence of translationally damaged proteins. Genetics 171(4):1477–1484

Chung KK, Thomas B, Li X, Pletnikova O, Troncoso JC, Marsh L, Dawson VL, Dawson TM (2004) S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. Science 304(5675):1328–1331

Chung CY, Seo H, Sonntag KC, Brooks A, Lin L, Isacson O (2005) Cell type-specific gene expression of midbrain dopaminergic neurons reveals molecules involved in their vulnerability and protection. Hum Mol Genet 14(13):1709–1725

Chung W-S, Clarke LE, Wang GX, Stafford BK, Sher A, Chakraborty C, Joung J, Foo LC, Thompson A, Chen C (2013) Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. Nature 504(7480):394–400

Chung W-S, Allen NJ, Eroglu C (2015) Astrocytes control synapse formation, function, and elimination. Cold Spring Harb Perspect Biol 7(9):a020370

Clarke LE, Liddelow SA, Chakraborty C, Münch AE, Heiman M, Barres BA (2018) Normal aging induces A1-like astrocyte reactivity. Proc Natl Acad Sci U S A 115(8):E1896–E1905

Çokal BG, Yurtdaş M, Güler SK, Güneş HN, Uçar CA, Aytaç B, Durak ZE, Yoldaş TK, Durak I, Çubukçu HC (2017) Serum glutathione peroxidase, xanthine oxidase, and superoxide dismutase activities and malondialdehyde levels in patients with Parkinson's disease. Neurol Sci 38(3): 425–431

Colangelo AM, Alberghina L, Papa M (2014) Astrogliosis as a therapeutic target for neurodegenerative diseases. Neurosci Lett 565:59–64

Cornford EM, Braun LD, Crane PD, Oldendorf WH (1978) Blood-brain barrier restriction of peptides and the low uptake of enkephalins. Endocrinology 103(4):1297–1303

Croisier E, Moran LB, Dexter DT, Pearce RK, Graeber MB (2006) Correction: Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. J Neuroinflammation 3(1):9

Członkowska A, Kohutnicka M, Kurkowska-Jastrzębska I, Członkowski A (1996) Microglial reaction in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced Parkinson's disease mice model. Neurodegeneration 5(2):137–143

D'Ambrosio R, Wenzel J, Schwartzkroin PA, McKhann GM, Janigro D (1998) Functional specialization and topographic segregation of hippocampal astrocytes. J Neurosci 18(12):4425–4438

Damier P, Hirsch E, Zhang P, Agid Y, Javoy-Agid F (1993) Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience 52(1):1–6

Datta I, Mishra S, Mohanty L, Pulikkot S, Joshi PG (2011) Neuronal plasticity of human Wharton's jelly mesenchymal stromal cells to the dopaminergic cell type compared with human bone marrow mesenchymal stromal cells. Cytotherapy 13(8):918–932

Datta I, Ganapathy K, Razdan R, Bhonde R (2018) Location and number of astrocytes determine dopaminergic neuron survival and function under 6-OHDA stress mediated through differential BDNF release. Mol Neurobiol 55(7):5505–5525

De CG, Gallet R, Cambier L (2017) Exosomal microRNA transfer into macrophages mediates cellular postconditioning. Circulation 136(2):200–214

Decressac M, Ulusoy A, Mattsson B, Georgievska B, Romero-Ramos M, Kirik D, Björklund A (2011) GDNF fails to exert neuroprotection in a rat α-synuclein model of Parkinson's disease. Brain 134(8):2302–2311

Deierborg T, Soulet D, Roybon L, Hall V, Brundin P (2008) Emerging restorative treatments for Parkinson's disease. Prog Neurobiol 85(4):407–432

Desai Bradaric B, Patel A, Schneider JA, Carvey PM, Hendey B (2012) Evidence for angiogenesis in Parkinson's disease, incidental Lewy body disease, and progressive supranuclear palsy. J Neural Transm 119(1):59–71

Desplats P, Lee H-J, Bae E-J, Patrick C, Rockenstein E, Crews L, Spencer B, Masliah E, Lee S-J (2009) Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. Proc Natl Acad Sci U S A 106(31):13010–13015

Dexter D, Wells F, Lee A, Agid F, Agid Y, Jenner P, Marsden C (1989) Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. J Neurochem 52(6):1830–1836

Djukic B, Casper KB, Philpot BD, Chin L-S, McCarthy KD (2007) Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J Neurosci 27(42):11354–11365

Do J, McKinney C, Sharma P, Sidransky E (2019) Glucocerebrosidase and its relevance to Parkinson disease. Mol Neurodegener 14:36

Duarte P, Jacques B, Ma W (2012) Prostaglandin E2 contributes to the synthesis of brain-derived neurotrophic factor in primary sensory neuron in ganglion explant cultures and in a neuropathic pain model. Exp Neurol 234(2):466–481

Durrenberger P, Filiou M, Moran L, Michael G, Novoselov S, Cheetham M, Clark P, Pearce R, Graeber M (2009) DnaJB6 is present in the core of Lewy bodies and is highly up-regulated in parkinsonian astrocytes. J Neurosci Res 87(1):238–245

Eggert K, Schlegel J, Oertel W, Würz C, Krieg J-C, Vedder H (1999) Glial cell line-derived neurotrophic factor protects dopaminergic neurons from 6-hydroxydopamine toxicity in vitro. Neurosci Lett 269(3):178–182

Emanuele M, Chieregatti E (2015) Mechanisms of alpha-synuclein action on neurotransmission: cell-autonomous and non-cell autonomous role. Biomol Ther 5(2):865–892

Farhy-Tselnicker I, van Casteren AC, Lee A, Chang VT, Aricescu AR, Allen NJ (2017) Astrocyte-secreted glypican 4 regulates release of neuronal pentraxin 1 from axons to induce functional synapse formation. Neuron 96(2):428–445.e413

Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. Trends Immunol 28(3):138–145

Fiacco TA, McCarthy KD (2018) Multiple lines of evidence indicate that gliotransmission does not occur under physiological conditions. J Neurosci 38(1):3–13
Finsterwald C, Magistretti PJ, Lengacher S (2015) Astrocytes: new targets for the treatment of neurodegenerative diseases. Curr Pharm Des 21(25):3570–3581
Flier J, Van Muiswinkel FL, Jongenelen CA, Drukarch B (2002) The neuroprotective antioxidant alpha-lipoic acid induces detoxication enzymes in cultured astroglial cells. Free Radic Res 36(6):695–699
Forno LS, DeLanney LE, Irwin I, Monte DD, Langston JW (1992) Chapter 36: Astrocytes and Parkinson's disease. In: Yu ACH, Hertz L, Norenberg MD, Syková E, Waxman SG (eds) Prog. brain res, vol 94. Elsevier, Amsterdam, pp 429–436. https://doi.org/10.1016/S0079-6123(08)61770-7
Freeman D, Cedillos R, Choyke S, Lukic Z, McGuire K, Marvin S, Burrage AM, Sudholt S, Rana A, O'Connor C (2013) Alpha-synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. PLoS One 8(4):e62143
Fu Y, Paxinos G, Watson C, Halliday GM (2016) The substantia nigra and ventral tegmental dopaminergic neurons from development to degeneration. J Chem Neuroanat 76(Pt B):98–107
Gabbott P, Bacon S (1996) Localisation of NADPH diaphorase activity and NOS immunoreactivity in astroglia in normal adult rat brain. Brain Res 714(1–2):135–144
Galea E, Feinstein DL, Reis DJ (1992) Induction of calcium-independent nitric oxide synthase activity in primary rat glial cultures. Proc Natl Acad Sci U S A 89(22):10945–10949
Ganapathy K, Datta I, Bhonde R (2019) Astrocyte-like cells differentiated from dental pulp stem cells protect dopaminergic neurons against 6-hydroxydopamine toxicity. Mol Neurobiol 56(6):4395–4413
Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B (2002) Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. J Neurochem 81(6):1285–1297
Gaoa L, Lia L, Ye J, Zhu X, Shen N, Zhanga X (2016) Identification of a novel mutation in PLA2G6 gene in a Chinese pedigree with familial cortical myoclonic tremor with epilepsy. Seizure 41:81–85
Gargioli C, Turturici G, Barreca MM, Spinello W, Fuoco C, Testa S, Feo S, Cannata SM, Cossu G, Sconzo G, Geraci F (2018) Oxidative stress preconditioning of mouse perivascular myogenic progenitors selects a subpopulation of cells with a distinct survival advantage in vitro and in vivo. Cell Death Dis 9(1):1
Gatto EM, Da Prat G, Etcheverry JL, Drelichman G (2019) Parkinsonisms and glucocerebrosidase deficiency: a comprehensive review for molecular and cellular mechanism of glucocerebrosidase deficiency. Brain Sci 9(2):30
Ge P, Dawson VL, Dawson TM (2020) PINK1 and Parkin mitochondrial quality control: a source of regional vulnerability in Parkinson's disease. Mol Neurodegener 15:20
Gegg M, Beltran B, Salas-Pino S, Bolanos J, Clark J, Moncada S, Heales S (2003) Differential effect of nitric oxide on glutathione metabolism and mitochondrial function in astrocytes and neurones: implications for neuroprotection/neurodegeneration? J Neurochem 86(1):228–237
Gerlach M, Ben-Shachar D, Riederer P, Youdim M (1994) Altered brain metabolism of iron as a cause of neurodegenerative diseases? J Neurochem 63(3):793–807
Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N (2010) Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 11(2):87–99
Gibb W, Lees A (1988) The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 51(6):745–752
Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R, Bunnage M, Brooks DJ, Svendsen CN, Heywood P (2003) Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. Nat Med 9(5):589–595
Ginns E, Choudary P, Suji ST, Martin B (1985) Gene mapping and leader polypeptide sequence of human glucocerebrosidase: implications for Gaucher disease. Proc Natl Acad Sci U S A 82(20):7101–7105

Gleiser C, Wagner A, Fallier-Becker P, Wolburg H, Hirt B, Mack AF (2016) Aquaporin-4 in astroglial cells in the CNS and supporting cells of sensory organs-a comparative perspective. Int J Mol Sci 17(9):1411

Gosejacob D, Dublin P, Bedner P, Hüttmann K, Zhang J, Tress O, Willecke K, Pfrieger F, Steinhäuser C, Theis M (2011) Role of astroglial connexin30 in hippocampal gap junction coupling. Glia 59(3):511–519

Granado N, Ares-Santos S, Moratalla R (2013) Methamphetamine and Parkinson's disease. Parkinson's Dis 2013:308052

Gray MT, Woulfe JM (2015) Striatal blood-brain barrier permeability in Parkinson's disease. J Cereb Blood Flow Metab 35(5):747–750

Griffiths P, Dobson B, Jones G, Clarke D (1999) Iron in the basal ganglia in Parkinson's disease: an in vitro study using extended X-ray absorption fine structure and cryo-electron microscopy. Brain 122(4):667–673

Grothe C, Timmer M (2007) The physiological and pharmacological role of basic fibroblast growth factor in the dopaminergic nigrostriatal system. Brain Res Rev 54(1):80–91

Gu X-L, Long C-X, Sun L, Xie C, Lin X, Cai H (2010) Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. Mol Brain 3:12–12

Hall J, Prabhakar S, Balaj L, Lai CP, Cerione RA, Breakefield XO (2016) Delivery of therapeutic proteins via extracellular vesicles: review and potential treatments for Parkinson's disease, glioma, and schwannoma. Cell Mol Neurobiol 36(3):417–427

Halliday GM, Stevens CH (2011) Glia: initiators and progressors of pathology in Parkinson's disease. Mov Disord 26(1):6–17

Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, Patel T, Piroyan A, Sokolsky M, Kabanov AV, Batrakova EV (2015) Exosomes as drug delivery vehicles for Parkinson's disease therapy. J Control Release 207:18–30

Hauser RA, Lyons KE, McClain T, Carter S, Perlmutter D (2009) Randomized, double-blind, pilot evaluation of intravenous glutathione in Parkinson's disease. Mov Disord 24(7):979–983

Hawkins V, Butt A (2013) TASK-1 channels in oligodendrocytes: a role in ischemia mediated disruption. Neurobiol Dis 55(100):87–94

Hayashi S, Wakabayashi K, Ishikawa A, Nagai H, Saito M, Maruyama M, Takahashi T, Ozawa T, Tsuji S, Takahashi H (2000) An autopsy case of autosomal-recessive juvenile parkinsonism with a homozygous exon 4 deletion in the parkin gene. Mov Disord 15(5):884–888

Hegarty SV, O'Keeffe GW, Sullivan AM (2014) Neurotrophic factors: from neurodevelopmental regulators to novel therapies for Parkinson's disease. Neural Regen Res 9(19):1708

Heneka M, Sastre L, Dumitrescu-Ozimek I, Dewachter I, Walter J, Van Leuven KF (2005) Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APP [V717I] transgenic mice. J Neuroinflammation 2:22

Heng BC, Lim LW, Wu W, Zhang C (2016) An overview of protocols for the neural induction of dental and oral stem cells in vitro. Tissue Eng Part B Rev 22:220–250

Henry AG, Aghamohammadzadeh S, Samaroo H, Chen Y, Mou K, Needle E, Hirst WD (2015) Pathogenic LRRK2 mutations, through increased kinase activity, produce enlarged lysosomes with reduced degradative capacity and increase ATP13A2 expression. Hum Mol Genet 24(21): 6013–6028

Hinson SR, Roemer SF, Lucchinetti CF, Fryer JP, Kryzer TJ, Chamberlain JL, Howe CL, Pittock SJ, Lennon VA (2008) Aquaporin-4-binding autoantibodies in patients with neuromyelitis optica impair glutamate transport by down-regulating EAAT2. J Exp Med 205(11):2473–2481

Hira K, Ueno Y, Tanaka R, Miyamoto N, Yamashiro K, Inaba T, Urabe T, Okano H, Hattori N (2018) Astrocyte-derived exosomes treated with a semaphorin 3A inhibitor enhance stroke recovery via prostaglandin D(2) synthase. Stroke 49(10):2483–2494

Hirano S, Asanuma K, Ma Y, Tang C, Feigin A, Dhawan V, Carbon M, Eidelberg D (2008) Dissociation of metabolic and neurovascular responses to levodopa in the treatment of Parkinson's disease. J Neurosci 28(16):4201

Hirrlinger J, König J, Dringen R (2002a) Expression of mRNAs of multidrug resistance proteins (Mrps) in cultured rat astrocytes, oligodendrocytes, microglial cells and neurones. J Neurochem 82(3):716–719

Hirrlinger J, Schulz JB, Dringen R (2002b) Glutathione release from cultured brain cells: multidrug resistance protein 1 mediates the release of GSH from rat astroglial cells. J Neurosci Res 69(3): 318–326

Hoddevik EH, Khan FH, Rahmani S, Ottersen OP, Boldt HB, Amiry-Moghaddam M (2017) Factors determining the density of AQP4 water channel molecules at the brain-blood interface. Brain Struct Funct 222(4):1753–1766

Huang J, Philbert MA (1995) Distribution of glutathione and glutathione-related enzyme systems in mitochondria and cytosol of cultured cerebellar astrocytes and granule cells. Brain Res 680(1–2):16–22

Hubbard JA, Binder DK (2017) Unaltered glutamate transporter-1 protein levels in aquaporin-4 knockout mice. ASN Neuro 9(1):1759091416687846

Hunot S, Dugas N, Faucheux B, Hartmann A, Tardieu M, Debré P, Agid Y, Dugas B, Hirsch EC (1999) FcεRII/CD23 is expressed in Parkinson's disease and induces, *In Vitro*, production of nitric oxide and tumor necrosis factor-α in glial cells. J Neurosci 19(9):3440–3447

Ibrahim A, Marbán E (2016) Exosmoes: fundamental biology and roles in cardiovascular physiology. Annu Rev Physiol 78:67–83

Iliff JJ, Nedergaard M (2013) Is there a cerebral lymphatic system? Stroke 44(6_Suppl_1):S93–S95

Iravani MM, Kashefi K, Mander P, Rose S, Jenner P (2002) Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience 110(1): 49–58

Itoh N, Itoh Y, Tassoni A, Ren E, Kaito M, Ohno A, Ao Y, Farkhondeh V, Johnsonbaugh H, Burda J (2018) Cell-specific and region-specific transcriptomics in the multiple sclerosis model: focus on astrocytes. Proc Natl Acad Sci U S A 115(2):E302–E309

Izrael M, Zhang P, Kaufman R, Shinder V, Ella R, Amit M, Itskovitz-Eldor J, Chebath J, Revel M (2007) Human oligodendrocytes derived from embryonic stem cells: effect of noggin on phenotypic differentiation in vitro and on myelination in vivo. Mol Cell Neurosci 34(3): 310–323

Janas AM, Sapoń K, Janas T, Stowell MH, Janas T (2016) Exosomes and other extracellular vesicles in neural cells and neurodegenerative diseases. Biochim Biophys Acta 1858(6): 1139–1151

Janda E, Isidoro C, Carresi C, Mollace V (2012) Defective autophagy in Parkinson's disease: role of oxidative stress. Mol Neurobiol 46(3):639–661

Janebodin K, Horst O, Ieronimakis N (2011) Isolation and characterization of neural crest-derived stem cells from dental pulp of neonatal mice. PLoS One 6(11):e27526

Jarmalavičiūtė A, Tunaitis V, Pivoraitė U, Venalis A, Pivoriūnas A (2015) Exosomes from dental pulp stem cells rescue human dopaminergic neurons from 6-hydroxy-dopamine–induced apoptosis. Cytotherapy 17(7):932–939

Jazwa A, Rojo AI, Innamorato NG, Hesse M, Fernández-Ruiz J, Cuadrado A (2011) Pharmacological targeting of the transcription factor Nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism. Antioxid Redox Signal 14(12):2347–2360

Jenner P (1993) Presymptomatic detection of Parkinson's disease. J Neural Transm Suppl 40:23–36

Jenner P, Olanow CW (1996) Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 47(6 Suppl 3):161S–170S

Jiang H, Wang J, Rogers J, Xie J (2017) Brain iron metabolism dysfunction in Parkinson's disease. Mol Neurobiol 54(4):3078–3101

Joe E-H, Choi D-J, An J, Eun J-H, Jou I, Park S (2018) Astrocytes, microglia, and Parkinson's disease. Exp Neurobiol 27(2):77

Junn E, Taniguchi H, Jeong BS, Zhao X, Ichijo H, Mouradian MM (2005) Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. Proc Natl Acad Sci U S A 102(27):9691–9696

Kannan R, Kuhlenkamp JF, Jeandidier E, Trinh H, Ookhtens M, Kaplowitz N (1990) Evidence for carrier-mediated transport of glutathione across the blood-brain barrier in the rat. J Clin Invest 85(6):2009–2013

Kaukua N, Shahidi M, Konstantinidou C, Dyachuk V, Kaucka M, Furlan A, An Z, Wang L (2014) Glial origin of mesenchymal stem cells in a tooth model system. Nature 513(7519):551–554

Kawajiri S, Saiki S, Sato S, Hattori N (2011) Genetic mutations and functions of PINK1. Trends Pharmacol Sci 32(10):573–580

Khasnavis S, Pahan K (2014) Cinnamon treatment upregulates neuroprotective proteins parkin and DJ-1 and protects dopaminergic neurons in a mouse model of Parkinson's disease. J Neuroimmune Pharmacol 9(4):569–581

Kim SU, de Vellis J (2005) Microglia in health and disease. J Neurosci Res 81(3):302–313

Kim KS, Kim JS, Park J-Y, Suh YH, Jou I, Joe E-H, Park SM (2013) DJ-1 Associates with lipid rafts by palmitoylation and regulates lipid rafts-dependent endocytosis in astrocytes. Hum Mol Genet 22(23):4805–4817

Kim J-M, Cha S-H, Choi YR, Jou I, Joe E-H, Park SM (2016) DJ-1 deficiency impairs glutamate uptake into astrocytes via the regulation of flotillin-1 and caveolin-1 expression. Sci Rep 6(1): 28823

Kinghorna KJ, Castillo-Quana JI (2016) Mitochondrial dysfunction and defects in lipid homeostasis as therapeutic targets in neurodegeneration with brain iron accumulation. Rare Dis 4(1): e1128616

Kinugawa K, Monnet Y, Lu L, Bekaert AJ, Théry C, Mallat Z, Hirsch EC, Hunot S (2013) MFGE8 does not orchestrate clearance of apoptotic neurons in a mouse model of Parkinson's disease. Neurobiol Dis 51:192–201

Knott C, Stern G, Kingsbury A, Welcher A, Wilkin G (2002) Elevated glial brain-derived neurotrophic factor in Parkinson's diseased nigra. Parkinsonism Relat Disord 8(5):329–341

Kojima R, Bojar D, Rizzi G, Hamri GC-E, El-Baba MD, Saxena P, Ausländer S, Tan KR, Fussenegger M (2018) Designer exosomes produced by implanted cells intracerebrally deliver therapeutic cargo for Parkinson's disease treatment. Nat Commun 9(1):1305

Kortekaas R, Leenders KL, van Oostrom JCH, Vaalburg W, Bart J, Willemsen ATM, Hendrikse NH (2005) Blood–brain barrier dysfunction in parkinsonian midbrain in vivo. Ann Neurol 57(2):176–179

Kostuk EW, Cai J, Iacovitti L (2018) Regional microglia are transcriptionally distinct but similarly exacerbate neurodegeneration in a culture model of Parkinson's disease. J Neuroinflammation 15(1):139

Koyano F, Matsuda N (2015) Molecular mechanisms underlying PINK1 and Parkin catalyzed ubiquitylation of substrates on damaged mitochondria. Biochim Biophys Acta 1853:2791–2796

Krencik R, Weick J, Liu Y, Zhang Z, Zhang S (2011) Specification of transplantable astroglial subtypes from human pluripotent stem cells. Nat Biotechnol 29(6):528–534

Kurkowska-Jastrzębska I, Wrońska A, Kohutnicka M, Członkowski A, Członkowska A (1999) The inflammatory reaction following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine intoxication in mouse. Exp Neurol 156(1):50–61

Lan Y-L, Zou S, Chen J-J, Zhao J, Li S (2016) The neuroprotective effect of the association of aquaporin-4/glutamate transporter-1 against Alzheimer's disease. Neural Plast 2016:4626593

Lanciotti A, Brignone M, Bertini E, Petrucci T, Aloisi F, Ambrosini E (2013) Astrocytes: emerging stars in leukodystrophy pathogenesis. Transl Neurosci 4(2):144–164

Lang AE, Gill S, Patel NK, Lozano A, Nutt JG, Penn R, Brooks DJ, Hotton G, Moro E, Heywood P, Brodsky MA, Burchiel K, Kelly P, Dalvi A, Scott B, Stacy M, Turner D, Wooten VG, Elias WJ, Laws ER, Dhawan V, Stoessl AJ, Matcham J, Coffey RJ, Traub M (2006a) Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. Ann Neurol 59(3):459–466

Lang AE, Langston JW, Stoessl AJ, Brodsky M, Brooks DJ, Dhawan V, Elias WJ, Lozano AM, Moro E, Nutt JG (2006b) GDNF in treatment of Parkinson's disease: response to editorial. Lancet Neurol 5(3):200–202

Ledesma MD, Galvan C, Hellias B, Dotti C, Jensen PH (2002) Astrocytic but not neuronal increased expression and redistribution of parkin during unfolded protein stress. J Neurochem 83(6):1431–1440

Lee H-C, Wei Y-H (2005) Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. Int J Biochem Cell Biol 37(4):822–834

Lee SW, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, Kim YJ, Kim KW (2003) SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. Nat Med 9(7): 900–906

Lee P, Lee G, Park H, Bang O, Joo I, Huh K (2006) The plasma alpha-synuclein levels in patients with Parkinson's disease and multiple system atrophy. J Neural Transm 113(10):1435–1439

Lee H-J, Suk J-E, Patrick C, Bae E-J, Cho J-H, Rho S, Hwang D, Masliah E, Lee S-J (2010a) Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem 285(12):9262–9272

Lee S, Varvel NH, Konerth ME, Xu G, Cardona AE, Ransohoff RM, Lamb BT (2010b) CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. Am J Pathol 177(5):2549–2562

Lee S, Yoon B-E, Berglund K, Oh S-J, Park H, Shin H-S, Augustine GJ, Lee CJ (2010c) Channel-mediated tonic GABA release from glia. Science 330(6005):790–796

Lee S, Lee WH, Lee MS, Mori K, Suk K (2012) Regulation by lipocalin-2 of neuronal cell death, migration, and morphology. J Neurosci Res 90(3):540–550

Lee MR, Ruby CL, Hinton DJ, Choi S, Adams CA, Young Kang N, Choi DS (2013) Striatal adenosine signaling regulates EAAT2 and astrocytic AQP4 expression and alcohol drinking in mice. Neuropsychopharmacology 38(3):437–445

Lee ML, Martinez-Lozada Z, Krizman EN, Robinson MB (2017) Brain endothelial cells induce astrocytic expression of the glutamate transporter GLT-1 by a Notch-dependent mechanism. J Neurochem 143(5):489–506

Lei XG, Cheng W-H, McClung JP (2007) Metabolic regulation and function of glutathione peroxidase-1. Annu Rev Nutr 27:41–61

Lev N, Barhum Y, Ben-Zur T, Melamed E, Steiner I, Offen D (2013) Knocking out DJ-1 attenuates astrocytes neuroprotection against 6-hydroxydopamine toxicity. J Mol Neurosci 50(3):542–550

Li A, Xi Q, Umstot ES, Bellner L, Schwartzman ML, Jaggar JH, Leffler CW (2008) Astrocyte-derived CO is a diffusible messenger that mediates glutamate-induced cerebral arteriolar dilation by activating smooth muscle Cell KCa channels. Circ Res 102(2):234–241

Li YH, Li JE, Liu WW (2017) Comparing the effect of neurotrophic factor induced MSCs (BMSC and DPSC) on the expression of myelin proteins Nogo-A and OMgp in a glaucoma rat model. Int J Clin Exp Med 10(3):4705–4713

Liddell JR, Hoepken HH, Crack PJ, Robinson SR, Dringen R (2006) Glutathione peroxidase 1 and glutathione are required to protect mouse astrocytes from iron-mediated hydrogen peroxide toxicity. J Neurosci Res 84(3):578–586

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung W-S, Peterson TC (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541(7638):481–487

Lin T-N, Wang P-Y, Chi S-I, Kuo J-S (1998) Differential regulation of ciliary neurotrophic factor (CNTF) and CNTF receptor α (CNTFRα) expression following focal cerebral ischemia. Mol Brain Res 55(1):71–80

Lin G, Lee P-T, Chen K, Mao D, Tan KL, Zuo Z, Lin W-W, Wang L, Bellen HJ (2018) Phospholipase PLA2G6, a parkinsonism-associated gene, affects Vps26 and Vps35, retromer function, and ceramide levels, similar to α-synuclein gain. Cell Metab 28(4):605–618.e6. https://doi.org/10.1016/j.cmet.2018.05.019

Lindahl M, Saarma M, Lindholm P (2017) Unconventional neurotrophic factors CDNF and MANF: structure, physiological functions and therapeutic potential. Neurobiol Dis 97:90–102

Lindström V, Gustafsson G, Sanders LH, Howlett EH, Sigvardson J, Kasrayan A, Ingelsson M, Bergström J, Erlandsson A (2017) Extensive uptake of α-synuclein oligomers in astrocytes

results in sustained intracellular deposits and mitochondrial damage. Mol Cell Neurosci 82:143–156

Liu C, Su C (2019) Design strategies and application progress of therapeutic exosomes. Theranostics 9(4):1015–1028

Liu S-Y, Wile DJ, Fu JF, Valerio J, Shahinfard E, McCormick S, Mabrouk R, Vafai N, McKenzie J, Neilson N (2018) The effect of LRRK2 mutations on the cholinergic system in manifest and premanifest stages of Parkinson's disease: a cross-sectional PET study. Lancet Neurol 17(4): 309–316

Loria F, Vargas JY, Bousset L, Syan S, Salles A, Melki R, Zurzolo C (2017) α-Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading. Acta Neuropathol 134(5):789–808

Lubos E, Loscalzo J, Handy DE (2011) Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 15(7):1957–1997

Luissint A-C, Artus C, Glacial F, Ganeshamoorthy K, Couraud P-O (2012) Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. Fluids Barriers CNS 9(1):23

Mader S, Brimberg L (2019) Aquaporin-4 water channel in the brain and its implication for health and disease. Cell 8(2):90

Manzoni C, Mamais A, Dihanich S, Abeti R, Soutar MPM, Plun-Favreau H, Giunti P, Tooze SA, Bandopadhyay R, Lewis PA (2013) Inhibition of LRRK2 kinase activity stimulates macroautophagy. Biochim Biophys Acta Mol Cell Res 1833(12):2900–2910

Martin R, Bajo-Graneras R, Moratalla R, Perea G, Araque A (2015) Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. Science 349(6249):730–734

Mashayekhi F, Mirzajani E, Naji M, Azari M (2010) Expression of insulin-like growth factor-1 and insulin-like growth factor binding proteins in the serum and cerebrospinal fluid of patients with Parkinson's disease. J Clin Neurosci 17(5):623–627

Mazzulli JR, Zunke F, Tsunemi T, Toker NJ, Jeon S, Burbulla LF, Patnaik S, Sidransky E, Marugan JJ, Sue CM (2016) Activation of β-glucocerebrosidase reduces pathological α-synuclein and restores lysosomal function in Parkinson's patient midbrain neurons. J Neurosci 36(29): 7693–7706

McGeer PL, Itagaki S, Boyes BE, McGeer EG (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38(8):1285

McLellan LI, Lewis AD, Hall DJ, Ansell JD, Wolf CR (1995) Uptake and distribution of N-acetylcysteine in mice: tissue-specific effects on glutathione concentrations. Carcinogenesis 16(9):2099–2106

Mead A, Logan A, Berry M, Leadbeater W (2014) Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem. PLoS One 9:e109305

Michinaga S, Koyama Y (2019) Dual roles of astrocyte-derived factors in regulation of blood-brain barrier function after brain damage. Int J Mol Sci 20(3):571

Mizuno Y, Suzuki K, Ohta S (1990) Postmortem changes in mitochondrial respiratory enzymes in brain and a preliminary observation in Parkinson's disease. J Neurol Sci 96(1):49–57

Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T (1994) Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci Lett 165(1):208–210

Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T (1996) Interleukin (IL)-1β, IL-2, IL-4, IL-6 and transforming growth factor-α levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. Neurosci Lett 211(1):13–16

Mogoanta L, Ciurea M, Pirici I, Margaritescu C, Simionescu C, Ion DA, Pirici D (2014) Different dynamics of aquaporin 4 and glutamate transporter-1 distribution in the perineuronal and perivascular compartments during ischemic stroke. Brain Pathol 24(5):475–493

Moran LB, Duke D, Deprez M, Dexter D, Pearce R, Graeber M (2006) Whole genome expression profiling of the medial and lateral substantia nigra in Parkinson's disease. Neurogenetics 7(1): 1–11

Moretto G, Walker DG, Lanteri P, Taioli F, Zaffagnini S, Xu RY (1996) Expression and regulation of glial-cell-line-derived neurotrophic factor (GDNF) mRNA in human astrocytes in vitro. Cell Tissue Res 286(2):257–262

Mori A, Hatano T, Inoshita T, Fukushima KS (2019) Parkinson's disease-associated iPLA2-VIA/PLA2G6 regulates neuronal functions and α-synuclein stability through membrane remodeling. Proc Natl Acad Sci U S A 116(41):20689–20699

Mullett SJ, Hinkle DA (2009) DJ-1 knock-down in astrocytes impairs astrocyte-mediated neuroprotection against rotenone. Neurobiol Dis 33(1):28–36

Mulligan SJ, MacVicar BA (2004) Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature 431(7005):195–199

Mythri RB, Venkateshappa C, Harish G, Mahadevan A, Muthane UB, Yasha T, Bharath MS, Shankar S (2011) Evaluation of markers of oxidative stress, antioxidant function and astrocytic proliferation in the striatum and frontal cortex of Parkinson's disease brains. Neurochem Res 36(8):1452–1463

Narendra D, Tanaka A, Suen D-F, Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 183(5):795–803

Navarro A, Boveris A (2009) Brain mitochondrial dysfunction and oxidative damage in Parkinson's disease. J Bioenerg Biomembr 41(6):517–521

Newman EA (2003) New roles for astrocytes: regulation of synaptic transmission. Trends Neurosci 26(10):536–542

Norenberg MD, Martinez-Hernandez A (1979) Fine structural localization of glutamine synthetase in astrocytes of rat brain. Brain Res 161(2):303–310

Offen D, Shtaif B, Hadad D, Weizman A, Melamed E, Gil-Ad I (2001) Protective effect of insulin-like-growth-factor-1 against dopamine-induced neurotoxicity in human and rodent neuronal cultures: possible implications for Parkinson's disease. Neurosci Lett 316(3):129–132

Ohlin KE, Francardo V, Lindgren HS, Sillivan SE, O'Sullivan SS, Luksik AS, Vassoler FM, Lees AJ, Konradi C, Cenci MA (2011) Vascular endothelial growth factor is upregulated by l-dopa in the parkinsonian brain: implications for the development of dyskinesia. Brain 134(8): 2339–2357

Osellame LD, Duchen MR (2013) Defective quality control mechanisms and accumulation of damaged mitochondria link Gaucher and Parkinson diseases. Autophagy 9(10):1633–1635

Paladino S, Conte A, Caggiano R, Pierantoni GM, Faraonio R (2018) Nrf2 pathway in age-related neurological disorders: insights into microRNAs. Cell Physiol Biochem 47(5):1951–1976

Park J-S, Koentjoro B, Veivers D, Mackay-Sim A, Sue CM (2014) Parkinson's disease-associated human ATP13A2 (PARK9) deficiency causes zinc dyshomeostasis and mitochondrial dysfunction. Hum Mol Genet 23(11):2802–2815

Park J-S, Blair NF, Sue CM (2015) The role of ATP13A2 in Parkinson's disease: clinical phenotypes and molecular mechanisms. Mov Disord 30(6):770–779

Parnetti L, Cicognola C, Eusebi P, Chiasserini D (2016) Value of cerebrospinal fluid α-synuclein species as biomarker in Parkinson's diagnosis and prognosis. Biomark Med 10(1):35–49

Pearce RK, Owen A, Daniel S, Jenner P, Marsden CD (1997) Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. J Neural Transm (Vienna) 104(6–7): 661–677

Pei X, Li Y, Zhu L, Zhou Z (2019) Astrocyte-derived exosomes suppress autophagy and ameliorate neuronal damage in experimental ischemic stroke. Exp Cell Res 382(2):111474

Perets N, Hertz S, London M, Offen D (2018) Intranasal administration of exosomes derived from mesenchymal stem cells ameliorates autistic-like behaviors of BTBR mice. Mol Autism 9:57

Perriot S, Mathias A, Perriard G, Canales M (2018) Human induced pluripotent stem cell-derived astrocytes are differentially activated by multiple sclerosis-associated cytokines. Stem Cell Rep 11(5):1199–1210

Petrova PS, Raibekas A, Pevsner J, Vigo N, Anafi M, Moore MK, Peaire A, Shridhar V, Smith DI, Kelly J, Durocher Y, Commissiong JW (2004) Discovering novel phenotype-selective neurotrophic factors to treat neurodegenerative diseases. Prog Brain Res 146:168–183

Pierre S-R, Lemmens MAM, Figueiredo-Pereira ME (2009) Subchronic infusion of the product of inflammation prostaglandin J2 models sporadic Parkinson's disease in mice. J Neuroinflammation 6:18–18

Power JH, Blumbergs PC (2009) Cellular glutathione peroxidase in human brain: cellular distribution, and its potential role in the degradation of Lewy bodies in Parkinson's disease and dementia with Lewy bodies. Acta Neuropathol 117(1):63–73

Qiao C, Yin N, Gu H-Y, Zhu J-L, Ding J-H, Lu M, Hu G (2016) Atp13a2 deficiency aggravates astrocyte-mediated neuroinflammation via NLRP3 inflammasome activation. CNS Neurosci Ther 22(6):451–460

Qu D, Rashidian J, Mount MP, Aleyasin H, Parsanejad M, Lira A, Haque E, Zhang Y, Callaghan S, Daigle M (2007) Role of Cdk5-mediated phosphorylation of Prx2 in MPTP toxicity and Parkinson's disease. Neuron 55(1):37–52

Ramaharobandro N, Borg J, Mandel P, Mark J (1982) Glutamine and glutamate transport in cultured neuronal and glial cells. Brain Res 244(1):113–121

Ramanadham S, Ali T, Ashley JW, Bone RN, Hancock WD (2015) Calcium-independent phospholipases A2 and their roles in biological processes and diseases. J Lipid Res 56(9): 1643–1668

Ramsey CP, Glass CA, Montgomery MB, Lindl KA, Ritson GP, Chia LA, Hamilton RL, Chu CT, Jordan-Sciutto KL (2007) Expression of Nrf2 in neurodegenerative diseases. J Neuropathol Exp Neurol 66(1):75–85

Ramsey CP, Tsika E, Ischiropoulos H, Giasson BI (2010) DJ-1 deficient mice demonstrate similar vulnerability to pathogenic Ala53Thr human alpha-syn toxicity. Hum Mol Genet 19(8): 1425–1437

Rannikko EH, Weber SS, Kahle PJ (2015) Exogenous α-synuclein induces toll-like receptor 4 dependent inflammatory responses in astrocytes. BMC Neurosci 16(1):1–11

Rappold PM, Tieu K (2010) Astrocytes and therapeutics for Parkinson's disease. Neurotherapeutics 7(4):413–423

Raps SP, Lai JC, Hertz L, Cooper AJ (1989) Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons. Brain Res 493(2):398–401

Reynolds AD, Glanzer JG, Kadiu I, Ricardo-Dukelow M, Chaudhuri A, Ciborowski P, Cerny R, Gelman B, Thomas MP, Mosley RL (2008) Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. J Neurochem 104(6):1504–1525

Rice ME, Russo-Menna I (1997) Differential compartmentalization of brain ascorbate and glutathione between neurons and glia. Neuroscience 82(4):1213–1223. https://doi.org/10.1016/S0306-4522(97)00347-3

Rice ME, Russo-Menna I (1998) Differential compartmentalization of brain ascorbate and glutathione between neurons and glia. Neuroscience 82(4):1213–1223

Riederer P, Sofic E, Rausch WD, Schmidt B, Reynolds GP, Jellinger K, Youdim MB (1989) Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. J Neurochem 52(2):515–520

Rieker C, Engblom D, Kreiner G, Domanskyi A, Schober A, Stotz S, Neumann M, Yuan X, Grummt I, Schütz G (2011) Nucleolar disruption in dopaminergic neurons leads to oxidative damage and parkinsonism through repression of mammalian target of rapamycin signaling. J Neurosci 31(2):453–460

Roberts RC, Roche JK, McCullumsmith RE (2014) Localization of excitatory amino acid transporters EAAT1 and EAAT2 in human postmortem cortex: a light and electron microscopic study. Neuroscience 277:522–540

Robinson MB, Jackson JG (2016) Astroglial glutamate transporters coordinate excitatory signaling and brain energetics. Neurochem Int 98:56–71

Ronnevi L-O (1978) Origin of the glial processes responsible for the spontaneous postnatal phagocytosis of boutons on cat spinal motoneurons. Cell Tissue Res 189(2):203–217

Saavedra A, Baltazar G, Santos P, Carvalho CM, Duarte EP (2006) Selective injury to dopaminergic neurons up-regulates GDNF in substantia nigra postnatal cell cultures: role of neuron-glia crosstalk. Neurobiol Dis 23(3):533–542

Salazar J, Mena N, Hunot S, Prigent A, Alvarez-Fischer D, Arredondo M, Duyckaerts C, Sazdovitch V, Zhao L, Garrick LM (2008) Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson's disease. Proc Natl Acad Sci U S A 105(47):18578–18583

Schipper HM, Song W, Zukor H, Hascalovici JR, Zeligman D (2009) Heme oxygenase-1 and neurodegeneration: expanding frontiers of engagement. J Neurochem 110(2):469–485

Seo B, Miura M, Gronthos S, Bartold P (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364(9429):149–155

Sharma S, Bandopadhyay R, Lashley T, Renton AEM, Kingsbury AE, Kumaran R, Kallis C, Vilariño-Güell C, O'Sullivan SS, Lees AJ, Revesz T, Wood NW, Holton JL (2011) LRRK2 expression in idiopathic and G2019S positive Parkinson's disease subjects: a morphological and quantitative study. Neuropathol Appl Neurobiol 37(7):777–790

Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, Jenner P, Marsden CD (1994) Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 36(3):348–355

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119(1): 7–35

Solano SM, Miller DW, Augood SJ, Young AB, Penney JB Jr (2000) Expression of α-synuclein, parkin, and ubiquitin carboxy-terminal hydrolase L1 mRNA in human brain: genes associated with familial Parkinson's disease. Ann Neurol 47(2):201–210

Solano RM, Casarejos MJ, Menéndez-Cuervo J, Rodriguez-Navarro JA, de Yébenes JG, Mena MA (2008a) Glial dysfunction in parkin null mice: effects of aging. J Neurosci 28(3):598–611

Solano RM, Casarejos MJ, Menéndez-Cuervo J, Rodriguez-Navarro JA, García de Yébenes J, Mena MA (2008b) Glial dysfunction in parkin null mice: effects of aging. J Neurosci 28(3): 598–611

Sorrentino ZA, Giasson BI, Chakrabarty P (2019) α-Synuclein and astrocytes: tracing the pathways from homeostasis to neurodegeneration in Lewy body disease. Acta Neuropathol 138(1):1–21

Souza JM, Giasson BI, Chen Q, Lee VM-Y, Ischiropoulos H (2000) Dityrosine cross-linking promotes formation of stable α-synuclein polymers implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. J Biol Chem 275(24): 18344–18349

Stavale LM, Soares ES, Mendonça MC, Irazusta SP, da Cruz Höfling MA (2013) Temporal relationship between aquaporin-4 and glial fibrillary acidic protein in cerebellum of neonate and adult rats administered a BBB disrupting spider venom. Toxicon 66:37–46

Stewart VC, Sharpe MA, Clark JB, Heales SJ (2000) Astrocyte-derived nitric oxide causes both reversible and irreversible damage to the neuronal mitochondrial respiratory chain. J Neurochem 75(2):694–700

Strokin M, Sergeeva M, Reiser G (2011) Proinflammatory treatment of astrocytes with lipopolysaccharide results in augmented Ca^{2+} signaling through increased expression of via phospholipase A2 (iPLA2). Am J Physiol Cell Physiol 300(3):C542–C549

Strokin M, Seburn KL, Cox GA, Martens KA, Reiser G (2012) Severe disturbance in the Ca^{2+} signaling in astrocytes from mouse models of human infantile neuroaxonal dystrophy with mutated Pla2g6. Hum Mol Genet 21(12):2807–2814

Stroomza MB, Barhum Y, Lev YS, Karpov O, Bulvik S, Melamed E (2009) Induction of adult human bone marrow mesenchymal stromal cells into functional astrocyte-like cells: potential for restorative treatment in Parkinson's disease. J Mol Neurosci 39:199–210

Su Z-z, Leszczyniecka M, Kang D-c, Sarkar D, Chao W, Volsky DJ, Fisher PB (2003) Insights into glutamate transport regulation in human astrocytes: cloning of the promoter for excitatory amino acid transporter 2 (EAAT2). Proc Natl Acad Sci U S A 100(4):1955–1960

Sun L, Shen R, Agnihotri SK, Chen Y, Huang Z, Bueler H (2018) Lack of PINK1 alters glia innate immune responses and enhances inflammation-induced, nitric oxide-mediated neuron death. Sci Rep 8(1):383

Sung JY, Park SM, Lee C-H, Um JW, Lee HJ, Kim J, Oh YJ, Lee S-T, Paik SR, Chung KC (2005) Proteolytic cleavage of extracellular secreted α-synuclein via matrix metalloproteinases. J Biol Chem 280(26):25216–25224

Tang KS, Suh SW, Alano CC, Shao Z, Hunt WT, Swanson RA, Anderson CM (2010) Astrocytic poly(ADP-ribose) polymerase-1 activation leads to bioenergetic depletion and inhibition of glutamate uptake capacity. Glia 58(4):446–457

Terada S, Ishizu H, Yokota O, Tsuchiya K, Nakashima H, Ishihara T, Fujita D, Uéda K, Ikeda K, Kuroda S (2003) Glial involvement in diffuse Lewy body disease. Acta Neuropathol 105(2): 163–169

Theodore S, Cao S, McLean PJ, Standaert DG (2008) Targeted overexpression of human α-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. J Neuropathol Exp Neurol 67(12):1149–1158

Théry C, Zitvogel L, Amigorena S (2002) Exosomes: composition, biogenesis and function. Nat Rev Immunol 2(8):569–579

Thiruchelvam M, Prokopenko O, Cory-Slechta DA, Richfield EK, Buckley B, Mirochnitchenko O (2005) Overexpression of superoxide dismutase or glutathione peroxidase protects against the paraquat + maneb-induced Parkinson disease phenotype. J Biol Chem 280(23):22530–22539

Thorburne SK, Juurlink BH (1996) Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. J Neurochem 67(3):1014–1022

Thumburu KK, Dhiman RK, Vasishta RK, Chakraborti A, Butterworth RF, Beauchesne E, Desjardins P, Goyal S, Sharma N, Duseja A, Chawla Y (2014) Expression of astrocytic genes coding for proteins implicated in neural excitation and brain edema is altered after acute liver failure. J Neurochem 128(5):617–627

Tokuda T, Qureshi M, Ardah M, Varghese S, Shehab S, Kasai T, Ishigami N, Tamaoka A, Nakagawa M, El-Agnaf O (2010) Detection of elevated levels of α-synuclein oligomers in CSF from patients with Parkinson disease. Neurology 75(20):1766–1770

Tomac A, Lindqvist E, Lin L-F, Ögren S, Young D, Hoffer B, Olson L (1995) Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. Nature 373(6512):335–339

Totterdell S, Meredith G (2005) Localization of alpha-synuclein to identified fibers and synapses in the normal mouse brain. Neuroscience 135(3):907–913

Tremblay M-E, Cookson MR, Civiero L (2019) Glial phagocytic clearance in Parkinson's disease. Mol Neurodegener 14(1):16

Trinh J, Amouri R, Duda JE, Morley JF, Read M, Donald A, Vilariño-Güell C, Thompson C, Szu Tu C, Gustavsson EK, Ben Sassi S, Hentati E, Zouari M, Farhat E, Nabli F, Hentati F, Farrer MJ (2014) A comparative study of Parkinson's disease and leucine-rich repeat kinase 2 p.G2019S parkinsonism. Neurobiol Aging 35(5):1125–1131

Tsacopoulos M, Magistretti PJ (1996) Metabolic coupling between glia and neurons. J Neurosci 16(3):877–885

Tseng K-Y, Danilova T, Domanskyi A, Saarma M, Lindahl M, Airavaara M (2017) MANF is essential for neurite extension and neuronal migration in the developing cortex. eNeuro 4(5): ENEURO.0214-17.2017

Tu P, Galvin JE, Baba M, Giasson B, Tomita T, Leight S, Nakajo S, Iwatsubo T, Trojanowski JQ, Lee VMY (1998) Glial cytoplasmic inclusions in white matter oligodendrocytes of multiple system atrophy brains contain insoluble α-synuclein. Ann Neurol 44(3):415–422

Tyzack G, Lakatos A, Patani R (2016) Human stem cell-derived astrocytes-specification and relevance for neurological disorders. Curr Stem Cell Rep 2:236–247

van der Pol E, Boing A, Harrison P, Sturk A (2012) Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev 64(3):676–705

van Muiswinkel FL, de Vos RA, Bol JG, Andringa G, Jansen Steur EN, Ross D, Siegel D, Drukarch B (2004) Expression of NAD(P)H:quinone oxidoreductase in the normal and Parkinsonian substantia nigra. Neurobiol Aging 25(9):1253–1262

Veen S, Sørensen DM, Holemans T, Holen HW, Palmgren MG (2014) Cellular function and pathological role of ATP13A2 and related P-type transport ATPases in Parkinson's disease and other neurological disorders. Front Mol Neurosci 7:48

Velayati A, Haung YW, Sidransky E (2010) The role of glucocerebrosidase mutations in Parkinson disease and Lewy body disorders. Curr Neurol Neurosci Rep 10(3):190–198

Venkateshappa C, Harish G, Mythri RB, Mahadevan A, Bharath MS, Shankar S (2012) Increased oxidative damage and decreased antioxidant function in aging human substantia nigra compared to striatum: implications for Parkinson's disease. Neurochem Res 37(2):358–369

Vilchez D, Ros S, Cifuentes D, Pujadas L, Valles J, Garcia-Fojeda B, Criado-García O, Fernandez-Sanchez E, Medraño-Fernández I, Domínguez J (2007) Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. Nat Neurosci 10(11): 1407–1413

Vitner EB, Dekel H, Zigdon H, Shachar T, Farfel-Becker T, Eilam R, Karlsson S, Futerman AH (2010) Altered expression and distribution of cathepsins in neuronopathic forms of Gaucher disease and in other sphingolipidoses. Hum Mol Genet 19(18):3583–3590

Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RLA, Kim J, May J, Tocilescu MA, Liu W, Ko HS, Magrané J, Moore DJ, Dawson VL, Grailhe R, Dawson TM, Li C, Tieu K, Przedborski S (2010) PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc Natl Acad Sci U S A 107(1):378–383

Vogiatzi T, Xilouri M, Vekre K, Stefanis L (2008) Wild type α-synuclein is degraded by chaperone-mediated autophagy and macroautophagy in neuronal cells. J Biol Chem 283(35):23542–23556

Waak J, Weber SS, Waldenmaier A, Görner K, Alunni-Fabbroni M, Schell H, Vogt-Weisenhorn D, Pham TT, Reumers V, Baekelandt V (2009) Regulation of astrocyte inflammatory responses by the Parkinson's disease-associated gene DJ–1. FASEB J 23(8):2478–2489

Wakabayashi K, Hayashi S, Yoshimoto M, Kudo H, Takahashi H (2000) NACP/α-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. Acta Neuropathol 99(1):14–20

Wang H, Atik A, Stewart T, Ginghina C, Aro P, Kerr KF, Seibyl J, Jennings D, Jensen PH, Marek K (2018) Plasma α-synuclein and cognitive impairment in the Parkinson's associated risk syndrome: a pilot study. Neurobiol Dis 116:53–59

Wong D, Dorovini-Zis K, Vincent SR (2004) Cytokines, nitric oxide, and cGMP modulate the permeability of an in vitro model of the human blood–brain barrier. Exp Neurol 190(2):446–455

Wu H, Friedman WJ, Dreyfus CF (2004) Differential regulation of neurotrophin expression in basal forebrain astrocytes by neuronal signals. J Neurosci Res 76(1):76–85

Wu N, Lu XQ, Yan HT, Su RB, Wang JF, Liu Y, Hu G, Li J (2008) Aquaporin 4 deficiency modulates morphine pharmacological actions. Neurosci Lett 448(2):221–225

Wu M, Ouyang Y, Wang Z, Zhang R, Huang P-H, Chen C, Li H, Li P, Quinn D, Dao M, Suresh S, Sadovsky Y, Huang TJ (2017) Isolation of exosomes from whole blood by integrating acoustics and microfluidics. Proc Natl Acad Sci U S A 114(40):10584–10589

Xiao N, Le Q-T (2016) Neurotrophic factors and their potential applications in tissue regeneration. Arch Immunol Ther Exp 64(2):89–99

Xin W, Schuebel KE, Jair K-w, Cimbro R, De Biase LM, Goldman D, Bonci A (2019) Ventral midbrain astrocytes display unique physiological features and sensitivity to dopamine D2 receptor signaling. Neuropsychopharmacology 44(2):344–355

Xu J, Yu S, Sun AY, Sun GY (2003) Oxidant-mediated AA release from astrocytes involves cPLA2 and iPLA2. Free Radic Biol Med 34(12):1531–1543

Xu L, Cao H, Xie Y, Zhang Y, Du M, Xu X, Ye R, Liu X (2019) Exosome-shuttled miR-92b-3p from ischemic preconditioned astrocytes protects neurons against oxygen and glucose deprivation. Brain Res 1717:66–73

Yang J, Li MX, Luo Y, Chen T, Liu J, Fang P, Jiang B, Hu ZL, Jin Y, Chen JG, Wang F (2013) Chronic ceftriaxone treatment rescues hippocampal memory deficit in AQP4 knockout mice via activation of GLT-1. Neuropharmacology 75:213–222

Yasuhara T, Shingo T, Muraoka K, Kameda M, Takeuchi A, Yano A, Nishio S, Matsui T, Miyoshi Y, Hamada H (2005) The differences between high and low-dose administration of VEGF to dopaminergic neurons of in vitro and in vivo Parkinson's disease model. Brain Res 1038(1):1–10

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32(18):6391–6410

Zecca L, Berg D, Arzberger T, Ruprecht P, Rausch WD, Musicco M, Tampellini D, Riederer P, Gerlach M, Becker G (2005) In vivo detection of iron and neuromelanin by transcranial sonography: a new approach for early detection of substantia nigra damage. Mov Disord 20(10):1278–1285

Zeevalk GD, Razmpour R, Bernard LP (2008) Glutathione and Parkinson's disease: is this the elephant in the room? Biomed Pharmacother 62(4):236–249

Zhang Y, Barres BA (2010) Astrocyte heterogeneity: an underappreciated topic in neurobiology. Curr Opin Neurobiol 20(5):588–594

Zhang H-Y, Wang N-D, Song N, Xu H-M, Shi L-M, Jiang H, Xie J-X (2013) 6-Hydroxydopamine promotes iron traffic in primary cultured astrocytes. Biometals 26(5):705–714

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34(36):11929–11947

Zhang Y, Sloan SA, Clarke LE, Caneda C, Plaza CA, Blumenthal PD, Vogel H, Steinberg GK, Edwards MS, Li G (2016a) Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. Neuron 89(1):37–53

Zhang Y, Tan F, Xu P, Qu S (2016b) Recent advance in the relationship between excitatory amino acid transporters and Parkinson's disease. Neural Plast 2016:8941327

Zhao Y, Haney MJ, Gupta R, Bohnsack JP, He Z, Kabanov AV, Batrakova EV (2014) GDNF-transfected macrophages produce potent neuroprotective effects in Parkinson's disease mouse model. PLoS One 9(9):e106867

Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Müller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 44(4): 601–607

Astroglial Pathology in Major Depressive Disorders: Metabolic and Molecular Aspects

Ajay Sarawagi, Unis Ahmad Bhat, Sanjana Sinha, Harish Iyer, Anant Bahadur Patel, and Arvind Kumar

Abstract

Major depressive disorder is a debilitating psychiatric condition that affects millions of people worldwide. Until recently, the neuronal component(s) of the brain was considered to be major players in the pathophysiology of depression. Recent advancements in Neuroscience research has suggested the involvement of glila cells in the biology of depression. Glial cell morphology, density, and glial markers were found to be altered in the brain of both, depressed subjects and preclinical models of depression. Moreover, perturbed neuron-glial communication in depressed subjects reveals a critical role of neurotransmitter cycling in the progression of the disease. Many ongoing studies are focused on uncovering the therapeutic potential of glial cells' manipulation in the treatment of depression. In this chapter, we have focused not only on the importance of glial cells in the efficient functioning of the brain but also on how neurons and glial cells communicate with each other and how their involvement could facilitate the progression of MDD. In addition, we have also reviewed the advancements in biophysical techniques like nuclear magnetic resonance (NMR) and positron emission tomography (PET) that are presently used to study different aspects of neuroenergetics. We conclude by proposing that further in-depth glia-centric studies pertaining to depression and associated psychiatric conditions would be radical for developing more efficient therapeutic strategies.

A. Sarawagi · A. B. Patel (✉) · A. Kumar (✉)
CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

Academy of Scientific and Innovative Research, Ghaziabad, India
e-mail: ajays@ccmb.res.in; abpatel@ccmb.res.in; akumar@ccmb.res.in

U. A. Bhat · S. Sinha · H. Iyer
CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India
e-mail: unis@ccmb.res.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_11

Keywords

Epigenetic · GABA · Glutamate · Neurometabolism · NMR

1 Introduction

The human brain is composed of billions of cells among which only neurons have the capacity to generate an electrical impulse (Azevedo et al. 2009). The rest of the cells are broadly called glia. Based on the distinct morphology and physiology, these cells are further categorized majorly in astrocytes, oligodendrocytes, microglia, ependymal cells in the central nervous system, and Schwann cells and satellite cells in the peripheral nervous system. Astrocytes, commonly known as astroglia, represent the most prevailing subtype of glial cells in the brain. For a long time, the main role of glia was thought to support the neurons. However, recent studies have established several roles of glia in regulating brain functions. These functions include the formation of the blood-brain barrier by supporting endothelial cells (Alvarez et al. 2013), regulation of blood flow during enhanced neuronal activity, bidirectional communications with neurons in regulating synaptogenesis, myelination (Nave 2010), neurotransmission, provision of substrates for neuronal energy metabolism (Pellerin et al. 1998), and maintenance of extracellular ion homeostasis (Simard and Nedergaard 2004).

The current understanding of functional and spatial relationship of astrocytes to neurons has led to the tripartite concept of the synaptic cleft. A single protoplasmic astrocyte can make astrocytic projections to many synapses, which are useful in the regulation of synaptic activity. In addition, astrocytes express the receptors for neurotransmitters and exercise tight control over the levels of neurotransmitters like GABA, glutamate, D-serine, and ATP in the synapse. Astrocytes actively clear neurotransmitters secreted by neurons from the synaptic cleft and recycle them back to neurons via glutamate-glutamine cycle. Besides this bidirectional communication with neurons, astrocyte also communicates with a subset of neighboring astrocyte networks. Moreover, recent studies have revealed novel, sophisticated, and more interesting role of astrocytes in complex brain functions like in memory and cognition.

Overall, there has been a major shift from a neuro-centric view of the brain to an equal appreciation for the importance of astrocytes and other nonneuronal cells. Considering the heterogeneity in the structure and function of astroglia, it is not surprising that these cells have been implicated in several neurological and neuropsychiatric conditions. Disruption of neuron-glia communication is involved in various psychiatric and neurodegenerative disorders including schizophrenia (Dietz et al. 2020), bipolar disorder (Keshavarz 2017), major depressive disorder (Czeh and Nagy 2018), and Alzheimer's disease (Patel et al. 2018; Soni et al. 2021). In this chapter, we describe evidence in support of abnormality in glial function in depression including altered neuronal and astroglial metabolic activity and pharmacological approaches targeting astroglial dynamics to improve neuronal functions.

1.1 Major Depressive Disorders

Major depressive disorder (MDD) is characterized by low mood, diminished interest in pleasurable activities, and/or suicidal ideation. It is one of the leading causes of distress and disability around the world (World Health Organization 2017). There are more than 300 million cases of depression worldwide (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators 2018). Despite decades of research, the pathophysiology of depression is not very clear yet. Numerous neuroimaging and postmortem reports have shown neuronal and astroglial atrophy in the prefrontal cortex (PFC) (Duman et al. 2016), anterior cingulate cortex (ACC), and hippocampus (HPC) (Elbejjani et al. 2015; Kim et al. 2018) of depressed subjects (Kempton et al. 2011; Schmaal et al. 2020). Several genetic factors (Howard et al. 2019), epigenetic changes such as histone acetylation or DNA methylation (Park et al. 2019), endocrine pathways such as elevated activity of hypothalamic-pituitary-adrenal (HPA) axis (Juruena et al. 2018), increased level of glucocorticoids, and decrease in the level of brain-derived neurotrophic factor (BDNF) in the prefrontal cortex and hippocampus of depressed subjects (Mondal and Fatima 2019) are implicated in the biology of the disorder.

The most prevalent hypothesis for depression posits that the depletion of monoamine neurotransmitters in synapse (Harmer et al. 2017; Perez-Caballero et al. 2019). Hence most of the antidepressant drugs are targeted to modulate the levels of these neurotransmitters. Recent studies in animal models and human subjects have shown abnormalities in glutamatergic and GABAergic systems with the pathophysiology of depression. Reduced expression of glutamate and GABA receptor subunits in different brain regions, altered neurotransmitter levels, impaired neurotransmission, and neurotransmitter cycling are reported in the literature (Lener et al. 2017). It is now becoming more evident that disruption of neuron-glia communication plays an important role in the etiology of depression (Abdallah et al. 2014; Duman et al. 2019; Mishra et al. 2020).

2 Glial Contributions to Neural Functions

Glial cells are the nonneuronal cells in the central nervous system (CNS) and peripheral nervous system (PNS) that do not communicate with electrical impulses (Fields et al. 2014). Astrocytes are broadly divided into two major groups: protoplasmic astrocytes in grey matter (present around neuronal cell bodies and synapses) and fibrous astrocytes in white matter (mostly localized to axons) (Kohler et al. 2021). It is envisioned that protoplasmic astrocytes differ in various regions of the brain. The spatiotemporal regulation of astrocyte development results in astrocytic heterogeneity in structure and function. The major functions of astroglia are described in subsequent sections.

2.1 Astrocytes as an Integral Part of the Neurovascular Unit and Synapse

Astrocytes regulate local blood flow for the supply of metabolic substrates and oxygen which support the energy requirement of neurons during intense neuronal activity via the release of vasoactive substances (Filosa et al. 2016). Individual astrocytes bidirectionally communicate with neurons and may ensheath thousands of synapses formed between many different neurons. This close spatial relationship and synaptic localization of astrocytes are vital for the regulation of synaptic transmission. In addition, astrocytes express receptors of numerous neurotransmitters, secrete a few of the gliotransmitters, and exercise tight control over the levels of neurotransmitters like GABA, glutamate, D-serine, and ATP in the synaptic space (Rothstein et al. 1994, 1996; Volterra and Meldolesi 2005). As such, astrocytes play a crucial role in neurotransmitter recycling via the glutamate-glutamine and GABA-glutamine cycle (**described in Sect. 4.2 Neurotransmitter Cycling**).

2.2 Astrocytes Contribute to Synapse Formation and Refinement

Astrocytes directly contribute to neurotransmission and at times prevent excitotoxicity in neurons. They also have been shown to contribute to synapse formation via secretion of extracellular factors like thrombospondins which have been demonstrated to promote glutamatergic synapses (Christopherson et al. 2005; Ullian et al. 2001). Astrocyte-specific gene expression profile has been shown to augment synapse assembly and continuous synapse refinement in an activity-dependent manner. For instance, deficits in astrocytic phagocytic pathways like MEGF10 and MERTK pathways result in attenuated synapses in visual circuits (Chung et al. 2015).

2.3 Astrocytes are Important Players in Neurological Disorders

The response of astrocytes to injury or disease is termed astrogliosis. It involves extensive proliferation of astrocytes that is reflected as elevated expression of GFAP and enhanced secretion of chondroitin sulfate proteoglycans, laminins, and fibronectin (Yuan and He 2013). Astrogliogenesis results in a glial scar which usually restricts neuronal injury to a limited area but has also been shown to be a major impediment in neural repair/axon regeneration post-injury. Increased population of reactive glia is the most common features in age-associated neurodegenerative diseases, viz., Alzheimer's disease, Parkinson's disease, Huntington's disease, stroke, amyotrophic lateral sclerosis, multiple sclerosis, and spinal cord injury (SCI) (Birch et al. 2014; Burda and Sofroniew 2014). Pathological transformation of astrocytes to cancer cells results in astrocytomas which are often resistant to standard antitumor therapeutics (Zong et al. 2015).

3 Techniques to Study Energy Requirement for Neural Function

There are several approaches to study neural function. These are mostly based on the energy requirement as well as the action potential of the cells. We will cover the techniques that are based on the energy requirement of neural cells. These are described below.

3.1 Positron Emission Tomography (PET)

PET is most commonly used for the measurement of local glucose and oxygen consumption, and blood flow in tissue. In this approach, ^{18}F-labeled glucose (2-fluorodeoxyglucose, ^{18}FDG) is used as a radio tracer. Due to absence of the OH group at second carbon in ^{18}FDG, the phosphorylated ^{18}FDG (^{18}FDG-6P) does not isomerize to fructose 6-phosphate and accumulates as ^{18}FDG-6P in the cells. The positron emitted by ^{18}FDG-6P is monitored by a sophisticated camera to reconstruct a three-dimensional image of the subject. The PET signal intensity is directly proportional to local glucose/oxygen consumption in tissue. PET imaging of microglia has grown over the last two decades due to development of radiopharmaceuticals specific to several molecular biomarkers of microglial activation. Many biological targets like 18 kDa translocator protein (TSPO), monoamine oxidase B (MAO-B), cyclooxygenase-1 and 2 (COX-1, COX-2), colony-stimulating factor 1 receptor (CSF1R), and purinergic P2X7 receptor (P2X7R) have been identified for the diagnosis and therapeutic purpose of neuroinflammation (Meyer et al. 2020). The commonly used ligands for PET measurements are isoquinoline carboxamide (^{11}C-PK11195), phenoxyarylacetamide derivatives labeled with carbon-11 (^{11}C-PBR28, ^{11}C-DAA1106) or fluorine-18 (^{18}F-FEPPA, ^{18}F-PBR06), imidazopyridines derivatives (^{11}C-CLINME, ^{11}C-DPA713, and ^{18}F-PBR111), and pyrazolopyrimidines derivatives (18F-DPA-714) (Dupont et al. 2017).

3.2 ^{13}C Nuclear Magnetic Resonance

The brain energy metabolism has been investigated extensively using ^{13}C-MRS together with an administration of ^{13}C-labeled metabolic substrates like glucose, acetate, and β-hydroxybutyrate (Gruetter et al. 2003; Patel et al. 2005; Sibson et al. 1998). As ^{13}C labeling at different carbon positions of glutamate, glutamine, GABA, and aspartate can be distinguished by ^{13}C-NMR, cell-type specific tricarboxylic acid (TCA) cycle fluxes and rates of different pathways can be measured by appropriate modeling of the ^{13}C turnover of neurometabolites (Patel et al. 2005, 2010; Tiwari et al. 2013).

Measurement of Neurometabolic Activity

In this approach ^{13}C-labeled glucose is administered intravenously, and kinetics of ^{13}C labeling of brain amino acids at different carbon positions is measured in vivo by ^{13}C-NMR spectroscopy (Gruetter et al. 2003). The metabolism of [1,6-$^{13}C_2$]glucose via glycolysis followed by TCA cycle labels glutamate-C4 (Glu_{C4}) in glutamatergic and GABAergic neurons (Fig. 1). In GABAergic neurons, Glu_{C4} is decarboxylated to $GABA_{C2}$ by glutamate decarboxylase (GAD). Glutamine-C4 (Gln_{C4}) gets labeled from Glu_{C4} and $GABA_{C2}$ through substrate cycling such as glutamate-glutamine and GABA-glutamine commonly known as neurotransmitter cycling. Further metabolism of Glu_{C4} and $GABA_{C2}$ in the corresponding TCA cycle labels aspartate-C2/C3 ($Asp_{C2/C3}$), $Glu_{C2/C3}$, and $GABA_{C3/C4}$ (Patel et al. 2018). The kinetics of ^{13}C label incorporation in different amino acids are analyzed using an appropriate model to determine the rate of glucose oxidation in the glutamatergic, GABAergic neurons, and rate of neurotransmitter cycling (Gruetter et al. 2003; Patel et al. 2005; Shen et al. 1999).

Measurement of Astroglial Activity

As acetate is selectively transported in astrocytes, it is used as a substrate to monitor astroglial metabolic activity. The metabolism of [2-^{13}C]acetate in the astrocytic TCA cycle labels Gln_{C4}. The labeling of $GABA_{C2}$ and Glu_{C4} occurs through glutamine-GABA and glutamine-glutamate cycling between astrocytes and respective neurons (Fig. 2) (Lebon et al. 2002; Patel et al. 2010; Tiwari et al. 2013). Hence, the kinetics of label incorporation into these amino acids provide an estimate rates of astrocytic TCA cycle and neurotransmitter cycling between neurons and astrocytes such as glutamate-glutamine cycle and GABA-glutamine cycle (Patel et al. 2010; Xin et al. 2010).

4 Brain Energy Metabolism

Although brain contributes only ~2% of total body weight, it utilizes ~20% of the total oxygen and 25% of glucose utilization, indicating the overwhelming energy demand of the brain (Sokoloff 1977). Energy budget estimates indicated that most of the energy utilized by the brain is used to support the processes associated with glutamate signaling such as postsynaptic glutamate receptors (50%) and action potential (20%) in the cerebral cortex (Attwell and Laughlin 2001; Howarth et al. 2012). Although glucose is the major energy substrate for the matured brain, monocarboxylates such as acetate, β-hydroxybutyrate, and lactate also contribute to a significant fraction of the brain energy under fasting and other nonphysiological conditions.

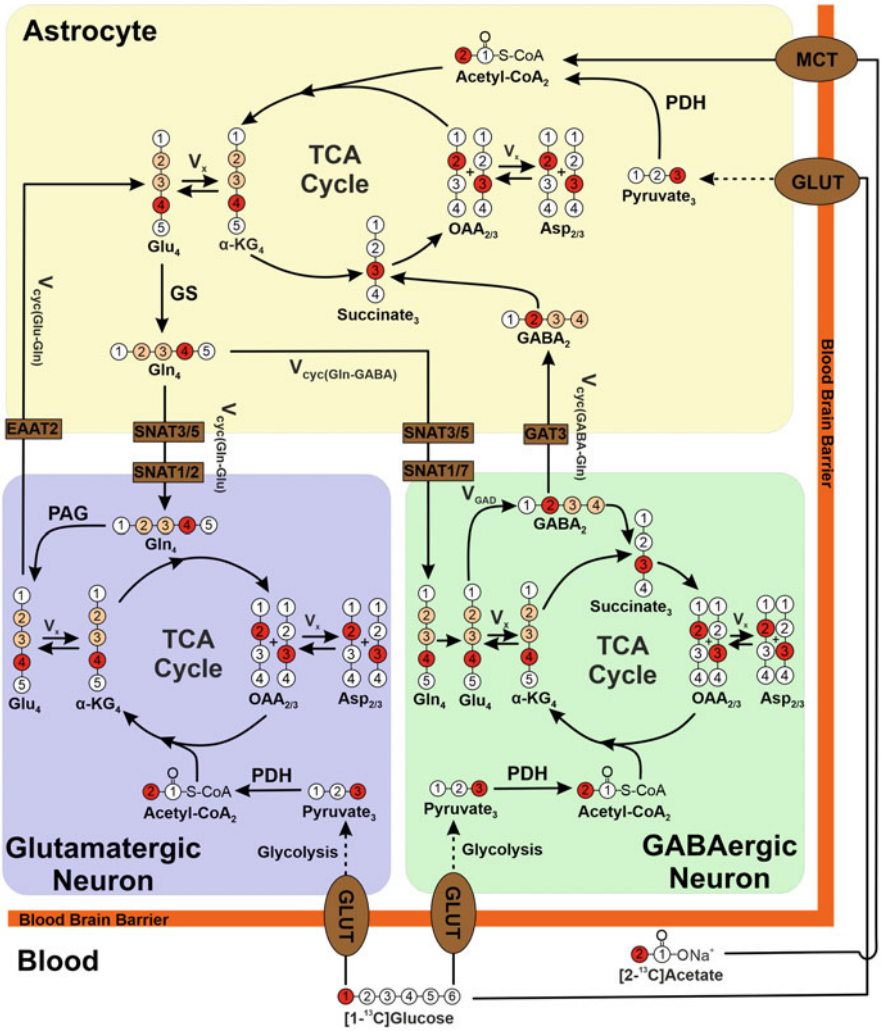

Fig. 1 Schematic of ^{13}C labeling of brain amino acids from [1-^{13}C]glucose and [2-^{13}C]acetate. Glucose is transported to neurons and astrocytes through GLUT; however, acetate is selectively transported in astrocytes through MCT. Glucose gets metabolized via glycolysis, and labels pyruvate$_{C3}$. Pyruvate dehydrogenase (PDH) complex converts pyruvate$_{C3}$ to acetyl-CoA$_{C2}$ that subsequently enters in TCA cycle and transfers ^{13}C label to Glu$_{C4}$ in all the three compartments. Glu$_{C4}$ gets converted to GABA$_{C2}$ by the action of the GAD enzyme in GABAergic neurons. Glu$_{C4}$ and GABA$_{C2}$ are released into synapses in the response of action potential from glutamatergic and GABAergic neurons, respectively. These neurotransmitters enter in astrocytes through EAAT2 and GAT3 transporter, respectively, and subsequently get converted in Gln$_{C4}$ by enzyme glutamine synthetase (GS). Gln is sent into the glutamatergic and GABAergic neurons via the members of the SNAT family of the transporter. Uptake of Glu$_{C4}$/GABA$_{C2}$ in astrocytes and release of Gln$_{C4}$ back to neurons is referred to as glutamate-glutamine and GABA-glutamine neurotransmitter cycling. Further metabolism of labeled intermediates of TCA cycle transfers label to α-KG$_{C2}$/α-KG$_{C3}$, Glu$_{C2}$/Glu$_{C3}$, Gln$_{C2}$/Gln$_{C3}$, and GABA$_{C3}$/GABA$_{C4}$ (labeled in sand color). *Abbreviations*: *α-KG$_4$* α-ketoglutarate-C4, *acetyl-CoA$_2$* acetyl co-enzymeA-C2, *Asp$_{2/3}$* aspartate-C2/C3, *EAAT2* excitatory amino acid transporter2, *GABA$_2$* γ-aminobutyric acid-C2, *GAT3* GABA transporter3, *GLUT*

4.1 Metabolic Activity of Neurons

^{13}C-NMR studies have indicated that ~70–85% of total energy is produced in the cerebral cortex by the neuronal mitochondrial TCA cycle and the remaining (~15–30%) by astroglia (Lebon et al. 2002; Patel et al. 2010). The GABAergic mitochondrial TCA cycle contributes to ~20% of the total neuronal TCA cycle in rats (Patel et al. 2005) and mice cerebral cortex (Tiwari et al. 2013). Moreover, these measurements have shown that rates of neurotransmitter cycling and oxidative glucose metabolism in neurons are stoichiometrically (1:1) coupled (Patel et al. 2004; Sibson et al. 1998) indicating neurotransmitter energetics is supported by oxidative glucose metabolism (Hyder et al. 2006; Magistretti et al. 1999). Neurons are incapable of de novo synthesis of glutamine as they lack glutamine synthetase enzymes. Hence, neurons are dependent on the astroglia to provide the precursor (glutamine) for the synthesis of glutamate and GABA (Patel et al. 2001). Additionally, astrocytes play an essential regulatory role in both glutamate-glutamine as well as GABA-glutamine neurotransmitter cycle (Bak et al. 2006; Benjamin and Quastel 1974).

4.2 Neurotransmitter Cycling

The cerebral TCA cycle is majorly responsible for the generation of energy and neurotransmitters glutamate and GABA in the brain. α-Ketoglutarate, a TCA cycle intermediate, acts as a precursor for glutamate (Hertz et al. 1999; Mason et al. 1995). Amino acid transaminase is responsible for the conversion of α-ketoglutarate to glutamate, and glutamate dehydrogenase regulates the reverse transformation. In response to an action potential, the glutamatergic neurons release glutamate into the synaptic cleft. After transmission of action potential to presynaptic neurons, neurotransmitter glutamate is taken up by astrocytes via excitatory amino acid transporters ($EAAT_{1/2}$). Astrocyte specific ATP-dependent glutamine synthetase (GS) regulates the transformation of glutamate to glutamine, which is transported to neurons via glutamine transporters (SNATs) (Edwards 2007). Glutamine is hydrolyzed to glutamate via phosphate-activated glutaminase (PAG) and repackaged into vesicles for the next release. This process is referred to as glutamate-glutamine neurotransmitter cycling (Bak et al. 2006; Chowdhury et al. 2007).

Fig. 1 (continued) glucose transporter, Glu_4 glutamate-C4, Gln_4 glutamine-C4, *GAD* glutamate decarboxylase, *GS* glutamine synthetase, *MCT* monocarboxylic acid transporter, $OAA_{2/3}$ oxaloacetate-C2/C3, *PAG* phosphate activated glutaminase, *PDH* pyruvate dehydrogenase, *SNAT* sodium-coupled neutral amino acid transporter, $V_{cyc(GABA-Gln)}$ GABA-glutamine cycling flux, $V_{cyc(Gln\text{-}GABA)}$ glutamine-GABA cycling flux, $V_{cyc(Glu\text{-}Gln)}$ glutamate-glutamine cycling flux, $V_{cyc(Gln\text{-}Glu)}$ glutamine-glutamate cycling flux, V_{GAD} glutamate decarboxylase flux, V_x flux of exchange between α-ketoglutarate-C4 and glutamate-C4, V_x exchange rate between oxaloacetate-C2/C3 and aspartate-C2/C3

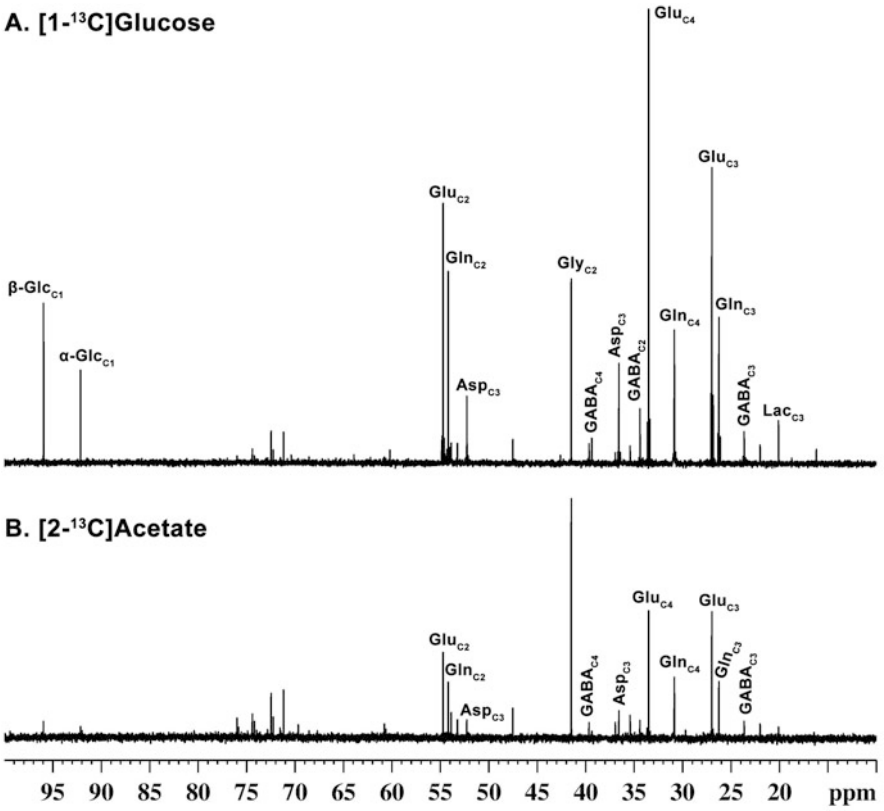

Fig. 2 ^{13}C NMR spectrum from the cerebral cortex of mouse infused with **A.** [1-^{13}C]Glucose and **B.** [2-^{13}C]Aacetate. Mice were infused with ^{13}C-labeled substrate for 90 min. Metabolites were extracted from the cerebral cortex, and ^{1}H-decoupled ^{13}C NMR spectrum was recorded at 150 MHz. As glucose is a physiological substrate for the brain, the incorporation of ^{13}C label into amino acids from [1-^{13}C]glucose is higher than with [2-^{13}C]acetate. Moreover, the intensity ratio of Gln_{C4}/Glu_{C4} is relatively more in mice infused with [2-^{13}C]acetate than [1-^{13}C]glucose, further confirms the astroglial-specific utilization of acetate. *Abbreviations*: Asp_{C3} aspartate-C3, $GABA_{C2}$ γ-aminobutyric acid-C2, $GABA_{C3}$ γ-aminobutyric acid-C3, $GABA_{C4}$ γ-aminobutyric acid-C4, Glu_{C2} glutamate-C2, Glu_{C3} glutamate-C3, Glu_{C4} glutamate-C4, Gln_{C2} glutamine-C2, Gln_{C3} glutamine-C3, Gln_{C4} glutamine-C4, $\beta\text{-}Glc_{C1}$ β-D-glucose-C1, $\alpha\text{-}Glc_{C1}$ α-D-glucose-C1, Lac_{C3} lactate-C3

Similarly, the substrate cycle involving GABA and glutamine (GABA-glutamine) occurs between GABAergic neurons and astrocytes (Bak et al. 2006). In this cycle, the released GABA into the synapse is taken up majorly by astrocytes via GABA transporters (GAT), wherein it gets converted to succinate via subsequent actions of GABA transaminase (GABA-T) and succinic semi-aldehyde dehydrogenase (SSADH), and ultimately converted to glutamine through TCA cycles (Patel et al. 2005). The glutamine thus formed is further transported to GABAergic neurons and converted to GABA by the successive action of glutaminase and glutamate

decarboxylase (GAD) (Patel et al. 2005; Tiwari et al. 2013). GAD exists in two isoforms in the CNS of mammals, GAD65 and GAD67. GAD65 is majorly enriched in nerve terminals and contributes to ~70% of total GAD protein while GAD67 is less abundant and distributed throughout the GABAergic neurons. GAD65 is believed to be involved in vesicular GABA synthesis and responsible for producing GABA in an activity-dependent manner, while GAD67 regulates GABA synthesis under normal brain activity (Soghomonian and Martin 1998).

5 Neuronal Metabolic Activity in Depression

N-Acetyl aspartate (NAA) is one of the strongest signals in ^{1}H-MRS, is exclusively localized in neurons, and is believed to reflect neuronal integrity and mitochondrial health (Paslakis et al. 2014). Reduced level of NAA has been reported in different brain regions including PFC (Jollant et al. 2016), ACC (Jarnum et al. 2011; Lewis et al. 2020), right frontal and parietal lobe (Kahl et al. 2020), and hippocampus (de Diego-Adelino et al. 2013) of depressed subjects. A lower level of NAA has also been reported in the hippocampus, nucleus accumbens (Cherix et al. 2020), and PFC (Mishra et al. 2018; Veeraiah et al. 2014) of CSDS and CUMS mouse models of depression. Reduced levels of NAA along with attenuated glutamate suggest a decrease in the viability of glutamatergic neurons and impairment of axon-myelin integrity in depression.

Availability of brain mitochondrial energetics related literature is limited for depression. A recent ^{18}F-fluoro-D-glucose positron emission tomography (FDG-PET) report showed decreased glucose metabolism in the left and right medial frontal gyri of the depressed subjects (Saito et al. 2017). ^{13}C MRS measurement along with an intravenous infusion of [1-^{13}C]glucose was performed to determine the impact of depression on neurotransmitter energetics in the occipital cortex of depressed subjects. The result from the study suggests that oxidative energy production from Glutamatergic neurons was decreased by 26% in depressed individuals (Abdallah et al. 2014). Another recent report using ^{1}H-[^{13}C]-nuclear magnetic resonance spectroscopy together with an infusion of [1,6-$^{13}C_2$]glucose showed a reduced rate of glucose oxidation in glutamatergic and GABAergic neurons in the prefrontal cortex (PFC) of CSDS (Veeraiah et al. 2014; Mishra et al. 2018) and CUMS mice (Mishra et al. 2020). More specifically, it was found that the rate of TCA cycle of glutamatergic and GABAergic neurons was decreased by 40% and 20%, respectively, in CUMS mice (Mishra et al. 2020). These reductions in neurometabolic flux lead to a large reduction in the rate of neuronal ATP synthesis.

6 Neuron-Glia Communication in Depression

Glutamine is mainly produced in glia from the reuptake of synaptic glutamate. It acts as a precursor for neurotransmitters, glutamate, and GABA and is involved in the substrate cycle glutamate-glutamine and GABA-glutamine (Patel et al. 2001). A

meta-analysis of MRS results of 16 studies has suggested a reduced level of glutamate or glutamate plus glutamine (Glx) in ACC and PFC of MDD patients (Luykx et al. 2012). It is thought that glutamatergic neurometabolic activity is decreased in depressed subjects (Arnone et al. 2015; Sarawagi et al. 2021). Additionally, it is suggested that alteration in astroglial pathology affects neurotransmission due to reduced expressions of glutamate and GABA transporter genes in depression models in cortical and subcortical regions of the brain. Decreased level of EAAT2 is reported in the hippocampus (Zhu et al. 2017) and PFC (Veeraiah et al. 2014) using the chronic unpredictable mild stress (CUMS) and chronic social defeat stress (CSDS) models of depression, respectively. Similarly, a reduced level of EAAT2 is also seen in the hippocampus in the learned helplessness model of depression (Zink et al. 2010). Moreover, decreased mRNA expression of glutamine synthetase (GS) is reported in several brain regions including PFC, ACC, dlPFC (Choudary et al. 2005), and locus coeruleus (Bernard et al. 2011). A decrease in the expression of GS enzyme points towards the compromised glial function potentially reduced neurotransmitter cycling. The reduced expression of EAAT2 suggests a decreased glutamate uptake capacity of astroglia that may lead to glutamate excitotoxicity in depression. In a recent study using CUMS model of depression, we have shown reduced rate of glutamate-glutamine cycling in the PFC of mice exhibiting depression-like phenotype (Mishra et al. 2020). Additionally, excitatory and inhibitory synaptic transmissions were reduced by ~40% in these mice. The evidence for reduced synaptic transmission in depression was further corroborated by reverse labeling (astrocytes to neurons) experiment using [2-^{13}C]acetate that shows decreased ^{13}C labeling of $GABA_{C2}$, Glu_{C4}, and Gln_{C4} in CUMS mice (Mishra et al. 2020). Most importantly, CUMS mice showed a reduction in ^{13}C-acetate metabolism which reflects the disrupted glial metabolic activity.

7 Glial Pathology in Major Depressive Disorder

Reduced astroglial densities have been reported in the postmortem studies of depressed patients (Cotter et al. 2001). The involvement of glia in the pathophysiology of depression has been studied by the use of glial specific markers such as GFAP, connexins, aquaporin4 (AQP4), vimentin, and various glutamate and GABA transporters. The density of each marker was determined using the immunohistochemical approach in formalin/paraformaldehyde fixed tissue blocks using specific antibodies (Miguel-Hidalgo et al. 2018; O'Leary et al. 2021; Rajkowska et al. 2013).

7.1 Astrocytic Pathology in MDD

Immunohistochemical Findings

Several studies have reported decreased glial density based on the GFAP expression in the dorsolateral prefrontal cortex (dlPFC) (Miguel-Hidalgo et al. 2000), ventromedial PFC (vmPFC) (Rajkowska et al. 2013), orbitofrontal cortex (OFC) (Miguel-

Hidalgo et al. 2010), ACC (Gittins and Harrison 2011), amygdala (Altshuler et al. 2010) and hippocampus (Cobb et al. 2016; Muller et al. 2001). Similar to GFAP, vimentin (VIM) is strongly expressed in cerebral astrocytes and is observed to follow a trend of decrease in astrocyte density in the gray and white matter of the dorsomedial PFC (O'Leary et al. 2021). Moreover, reduced glial density is reported based on the connexin-30 and connexin-43 expression in the pre-limbic cortex and OFC of a rat model of depression (Miguel-Hidalgo et al. 2018) as well as in the postmortem brain tissues of the depressed subjects (Fig. 3) (Miguel-Hidalgo et al. 2014). Additionally, glial density measured by the expression of AQP4 is found to be decreased in the PFC of depressed subjects (Table 1) (Rajkowska et al. 2013).

Studies Involving mRNA and Protein Level

GFAP protein has been found to be decreased in the OFC (Miguel-Hidalgo et al. 2010), frontal cortex (Johnston-Wilson et al. 2000), dlPFC (Si et al. 2004) and lateral cerebellum (Fatemi et al. 2004) in the postmortem brain of depressed subjects. Reduced GFAP mRNA expression in the PFC has also been reported in VPFC of depressed subjects (Rajkowska et al. 2018) as well as in the chronic unpredictable stress (CUS) model of depression (Banasr et al. 2010). Moreover, GFAP was found to be downregulated in both mRNA and protein levels in the pontine locus coeruleus (PLC) region of the brain in depressed subjects (Chandley et al. 2013). Similarly, protein levels of connexin43 were found to be downregulated in OFC of depressed subjects (Miguel-Hidalgo et al. 2014) and in the PFC and hippocampus of rat and mice brain, respectively (Huang et al. 2019; Sun et al. 2012). Moreover, the levels of connexin30 and connexin43 mRNA were reported to be decreased in the motor cortex, visual cortex, medio-dorsal thalamus and caudate nucleus. Interestingly, the connexin30 mRNA level was reported as upregulated in the cerebellum (Table 2) (Nagy et al. 2017).

A decrease in the expression and density of immunoreactive GFAP suggests a lack of reactive astrocytes and loss of astrocytes in MDD (Kim et al. 2018; O'Leary and Mechawar 2021). Evidence from different postmortem studies suggest a decrease in the communication of astrocytes with other cell types in MDD. Moreover, the downregulation of AQP4 and connexin proteins in depression suggests decreased vascular contacts and fewer gap junctions involving glial cells. Hence, astrocytes pathophysiology is a hallmark of major depressive disorder.

7.2 Oligodendrocyte Pathology in MDD

Oligodendrocyte (OL) ensheaths unmyelinated fibers, thereby isolating different neuronal pathways (Rajkowska and Miguel-Hidalgo 2007; Zhou et al. 2021). A decreased OL density has been reported in layer VI in PFC (Uranova et al. 2004). This points to the atrophy of pyramidal neurons in layer VI. Reduced myelination in the medial PFC along with downregulation of myelin related genes, myelin oligodendrocyte glycoprotein, ermin, and myelin basic protein has been reported in the CSDS mouse model of depression (Fig. 3) (Lehmann et al. 2017). Additionally, a

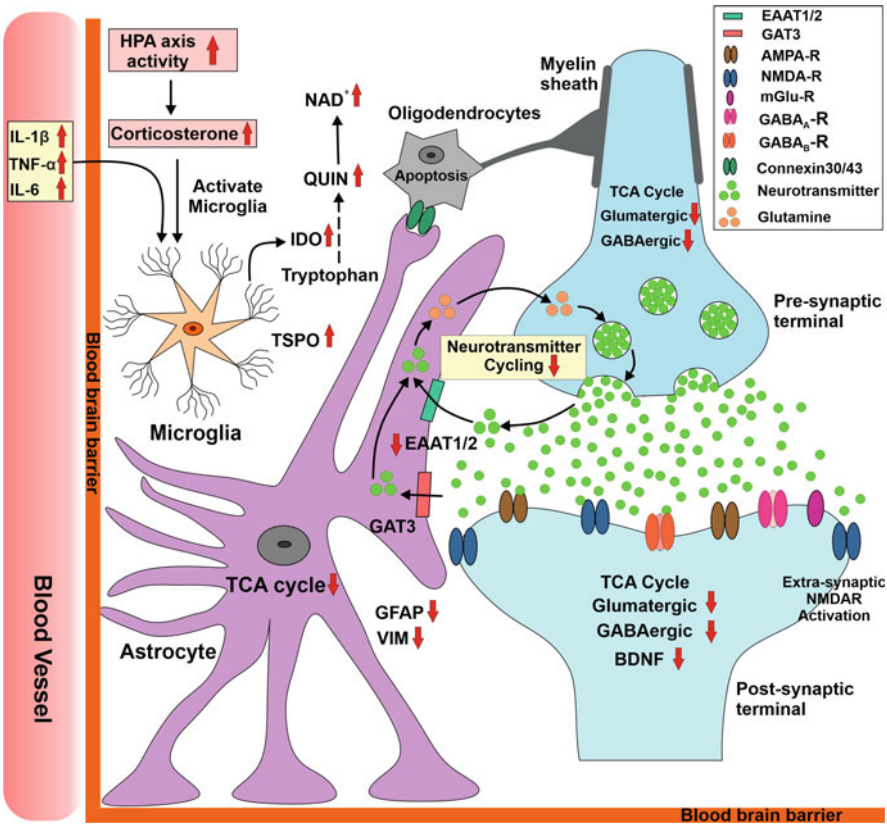

Fig. 3 Neuroglial pathology in major depressive disorder. Elevated activity of the HPA axis results in increased levels of corticosterone in blood and CSF. Increased level of cortisol along with a high level of peripheral inflammatory cytokines such as IL-1β, TNF-α and IL-6 leads to the activation of microglia cells (increased expression of TSPO in PET studies). Activated microglial cells accompany to increase in levels of proinflammatory cytokines that leads to activation of IDO enzyme, and diverts tryptophan digestion to kynurenine pathway. This leads to an accumulation of QUIN and NAD^+ thereby oxidative stress and neurotoxicity. QUIN acts as an NMDA receptor agonist and binds with NMDA receptors located on the presynaptic membrane, leading to increased glutamate release. Moreover, GABAergic interneurons lose their control (decrease level of GABA) over the glutamatergic neurons that results in excessive glutamate release. Glial cells lose their integrity and morphology as shown in decreased levels of GFAP, VIM, and loss of astroglial end feet (decreased expression of aquaporin-4) and connection (decreased level of Connexin30/43) with surrounding glial cells. The expression of excitatory amino acid transporter (EAAT1/2) also goes down, leading to decreased glutamate uptake from the synaptic cleft. The increased glutamate level in the synaptic cleft leads to over activation of intrasynaptic ionotropic receptors including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate receptors (NMDA) that may potentially lead to excitotoxicity. Additionally, spillover glutamate also binds with extra-synaptic NMDA receptors that suppresses BDNF-TrkB pathway and results in decreased synaptogenesis. Moreover, inflammatory cytokines induce apoptosis in oligodendrocytes resulting in demyelination. Additionally, the rates of astroglial, glutamatergic, and GABAergic neuronal TCA cycle are reported to be down in depression. Moreover, the reduced rates of neurotransmitter cycling involving glutamate-glutamine and GABA-glutamine are reported in the pathology of depression.

Table 1 Morphometric analysis of astrocyte biomarkers in major depressive disorder

SN	Subject	Control/ MDD	Marker	Brain region	Density	Study
1.	Human	10/10	GFAP	CN	↓	O'Leary et al. (2021)
			GFAP	MDT	↓	
			VIM	DMPFC	↓	
2.	Rats	6/6	CX-43, CX-30	PC, OFC	↓	Miguel-Hidalgo et al. (2018)
3.	Human, rats	8/8,10/10	GFAP	VPFC	↓	Rajkowska et al. (2018)
4.	Human	17/17	GFAP	HPC	↓	Cobb et al. (2016)
5.	Human	20/36	CX-43	OFC	NS	Miguel-Hidalgo et al. (2014)
6.	Human	13/13	AQP4	PFC	↓	Rajkowska et al. (2013)
7.	Human	9/7	GFAP	ACC	↓	Gittins and Harrison (2011)
8.	Human	14/11	GFAP	Amygdala	↓	Altshuler et al. (2010)
9.	Human	13/23	GFAP	OFC	↓	Miguel-Hidalgo et al. (2010)
10.	Human	19/20	GFAP	PLC	↓	Chandley et al. (2013)
11.	Human	16/15	GFAP	HPC	↓	Muller et al. (2001)
12.	Human	15/14	GFAP	DLPFC	↓	Miguel-Hidalgo et al. (2000)

Abbreviations: *ACC* anterior cingulate cortex, *AQP4* aquaporin-4, *CX-30* connexin-30, *CX-43* connexin-43, *CN* caudate nucleus, *DMPFC* dorsomedial prefrontal cortex, *DLPFC* dorsolateral prefrontal cortex, *GFAP* glial fibrillary acidic protein, *HPC* hippocampus, *MDT* mediodorsal thalamus, *NS* not significant, *OFC* orbitofrontal cortex, *PFC* prefrontal cortex, *PC* prelimbic cortex, *PLC* pontine locus coeruleus, *VPFC* ventral prefrontal cortex, *VIM* vimentin

decrease in total volume and length of myelinated nerve fiber has been reported in the CUS model of rats (Gao et al. 2017). These arguments point towards a possibility of loss in glial population in depression.

Fig. 3 (continued) Abbreviations: *AMPA-R* α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, *BDNF* brain-derived neurotrophic factor, *EAAT* excitatory amino acid transporter, *GAT* GABA transporter, $GABA_A$*-R* ionotropic GABA receptor, $GABA_B$*-R* metabotropic GABA receptor, *GFAP* glial fibrillary acidic protein, *IL-1β* interleukin-1beta, *IL-6* interleukin-6, *IDO* indoleamine 2,3-deoxygenase, *mGLU-R* metabotropic glutamate receptor, *NMDA-R* *N*-methyl D-aspartate receptor, NAD^+ nicotinamide adenine dinucleotide, *QUIN* quinolinic acid, *TCA* tricarboxylic acid cycle, *TNF-α* tumor necrosis factor-alpha, *TSPO* 18 kDa-translocator protein, *VIM* vimentin

Table 2 Dysregulation in the expression of astrocyte biomarkers at the level of mRNA and protein in MDD

SN	Subject	Control/ MDD	Marker	Brain region	mRNA	Protein	Study
1.	Mice	14/12	CX-43, CX-30	mPFC, HPC	NA	↓	Huang et al. (2019)
2.	Human	8/8	GFAP	VPFC	↓	NA	Rajkowska et al. (2018)
3.	Human	22/22	CX-43, CX-30	MC, VC, CB, CN, MDT	↓	NA	Nagy et al. (2017)
4.	Human	20/36	CX-43	OFC	NA	↓	Miguel-Hidalgo et al. (2014)
5.	Human	19/20	GFAP	PLC	↓	↓	Chandley et al. (2013)
6.	Rats	NA	CX-43	PFC	↓	↓	Sun et al. (2012)
7.	Human	9/12	AQP4, SLC1A2, SLC1A3, GS	LC	↓	NA	Bernard et al. (2011)
8.	Human	13/23	GFAP	LOFC	NA	↓	Miguel-Hidalgo et al. (2010)
9.	Rats	16/16	GFAP	PC	↓	NA	Banasr et al. (2010)
10.	Human	13/16	GS	PFC	↓	NA	Sequeira et al. (2009)
11.	Human	7/9	SLC1A2, SLC1A3, GS	ACC, DLPFC	↓	NA	Choudary et al. (2005)
12.	Human	15/15	GFAP	Lateral CB	NA	↓	Fatemi et al. (2004)
13.	Human	15/15	GFAP	DLPFC	NA	↓	Si et al. (2004)
14.	Human	19/23	GFAP	FC	NA	↓	Johnston-Wilson et al. (2000)

Abbreviations: *ACC* anterior cingulate cortex, *AQP4* aquaporin-4, *CX-30* connexin-30, *CX-43* connexin-43, *CN* caudate nucleus, *CB* cerebellum, *DMPFC* dorsomedial prefrontal cortex, *DLPFC* dorsolateral prefrontal cortex, *GFAP* glial fibrillary acidic protein, *GS* glutamine synthetase, *FC* frontal cortex, *HPC* hippocampus, *LOFC* left orbitofrontal cortex, *LC* locus coeruleus, *MC* motor cortex, *MDT* mediodorsal thalamus, *NA* not applicable, *OFC* orbitofrontal cortex, *PFC* prefrontal cortex, *PC* prelimbic cortex, *PLC* pontine locus coeruleus, *SLC1A* solute carrier family 1 member, *VPFC* ventral prefrontal cortex, *VC* visual cortex, *VIM* vimentin

7.3 Neuroinflammation in Major Depressive Disorder

Astrocytes along with other glial cells such as oligodendrocytes and microglia protect the brain from pathogens, injury, and destructive immune cells from the periphery. Neuroinflammation is defined as an increase in the level of

pro-inflammatory molecules, activation of resident microglia, and increase in the permeability of blood-brain barrier (BBB) (Troubat et al. 2021). Microglial cells are the immune cells of the CNS and get activated during stress, injury, and pathogen invasion. The activated microglia release pro-inflammatory molecules such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1beta (IL-1β), and recruit other immune cells. Neuroinflammation triggered by stress and other conditions causes dysregulation in different pathways in MDD such as kynurenine pathway alteration and HPA axis dysfunction which are explained below.

Dysfunction in Hypothalamus-Pituitary-Adrenal (HPA) Axis

Cortisol released from the HPA axis has several central and peripheral roles in metabolic, cardiovascular, and central nervous systems (Dedovic et al. 2009). The dysfunction in HPA axis due to stress and anxiety disrupts the negative feedback mechanism that leads to excessive release of cortisol (Holsboer 2000). Cortisol binds to the glucocorticoid receptor (GR) of the hippocampus and regulates its function. Thus, an impaired GR can lead to HPA axis disruption as shown in transgenic mice having forebrain-specific disruption of GR (Boyle et al. 2005). The role of GR was further supported in a pharmacological study involving the administration of RU486, a GR antagonist that prevents microglial activation in stressed mice (Nair and Bonneau 2006: 139). These findings suggest a possible link between activation of microglia and HPA axis dysfunction (Fig. 3).

Dysfunction in Kynurenine Pathway

The kynurenine pathway dominates in the liver and also occurs in CNS where it synthesizes bioactive compounds. The pro-inflammatory molecules increase the expression of indoleamine 2,3-dioxygenase 1 (IDO) which catabolizes tryptophan to kynurenine (KYN). It has two facets: *Neuroprotective* and *Neurotoxic* (Colpo et al. 2019). The neuroprotective pathway is regulated by kynurenine aminotransferases (KATs), an astrocyte specific enzyme, which converts KYN into kynurenic acid. It acts as an NMDA receptor antagonist and has an antidepressant-like action. On the other hand, kynurenine-3-monooxygenase (KMO), a microglia-specific enzyme produces toxic molecules like quinolinic acid (an agonist of NMDA receptor) and NAD+ under inflammatory condition, and contributes to neurotoxicity (Troubat et al. 2021). A preclinical study conducted in rats following LPS administration has shown an increase in IDO and KMO activity in the brain suggesting activation of neurotoxic pathway under inflammatory condition (Connor et al. 2008). This finding was further supported by the finding of lack of depressive like behavior in $KMO^{-/-}$ mice (Parrott et al. 2016). Moreover, a decrease in the level of kynurenic acid in the amygdala, and an increase in 3-hydroxykynurenine in the striatum of mouse model of depression suggest that stress can lead to the toxicity in the brain regions involved in depression (Fig. 3) (Laugeray et al. 2016).

Microglial Activation

Multiple reports includes an increase in the levels of peripheral cytokines in depressed subjects (Enache et al. 2019), and an association between central inflammation and depression-like symptoms (Felger et al. 2020; Zhang et al. 2016) supports the microglial dysregulation hypothesis for MDD etiology. A recent study measured the total distribution volume of TSPO (a marker for microglial activation, TSPO-V_T) using [^{18}F]-FEPPA PET imaging in MDD subjects. The TSPO-V_T was found to be elevated in the neocortical gray matter, white matter, frontal cortex, temporal cortex, and hippocampus in MDD relative to the control subjects (Li et al. 2018). Similarly, TSPO-V_T was found to be 25% and 15% elevated in the subgenual prefrontal cortex and ACC, respectively, measured using ^{11}C-PBR28 in unmedicated MDD subjects (Richards et al. 2018). In addition, TSPO was found to be robustly upregulated in ACC (67%) and insula (24%) region of the depressed subjects with suicidal ideation (Holmes et al. 2018). Moreover, TSPO level increases with the disease progression in unmedicated patients (Fig. 3) (Setiawan et al. 2018). These findings strongly suggest neuroinflammation, specifically microglial activation in major depressive episodes.

There are some inconsistencies in the literature, which raises further questions. A previous report of microglia PET imaging using ^{11}C-PRB28 did not show any significant difference in TSPO levels in the frontal cortex and gray matter regions of depressed subjects as compared with healthy controls (Hannestad et al. 2013). Moreover, the use of TSPO as an indirect measure of microglia activation has been challenged by a recent report suggesting that elevated TSPO in humans may reflect the local myeloid cell proliferation or increased recruitment of monocytes rather than activation of microglia (Owen et al. 2017).

8 Manipulation of Glial Function as a Therapeutic Strategy for Neuropsychiatric Disorders

Therapeutic interventions for MDD are limited and are often insufficient to resolve depressive state completely. Therefore, new and effective treatment strategies are warranted. In pursuit of such novel and more effective treatments for MDD, preclinical studies from the past decade are augmenting the targeting of astrocytes as an alternative strategy. However, the present understanding of the mechanism of commonly prescribed pharmacological antidepressants and interventions like deep brain stimulation (DBS) therapy and electroconvulsive therapy (ECT) on astrocytes is limited. The following section discusses the modulation of astrocyte activities in depression.

8.1 Classical Antidepressants Modulate Astrocytic Activity

Pharmacological management of depression with serotonin specific reuptake inhibitors (fluoxetine, paroxetine, citalopram, and sertraline) has been demonstrated to manipulate signaling mechanisms both in neurons and astrocytes (Hertz et al. 2015; Rivera and Butt 2019). Like neurons, astrocytes express various cell surface receptors including 5-HT2B, 5-HT2A and 5-HT2C receptors. Expression of 5-HT2B receptors has been shown to mediate the effects of SSRIs (Hertz et al. 2012, 2015). Acute fluoxetine treatment induces ERK1/2 phosphorylation in a 5-HT2B receptor-dependent manner in astrocytes (Li et al. 2009). Phosphorylated ERK1/2 translocates to the nucleus and regulates gene expression. Similarly, the administration of fluoxetine reverses the effect of chronic mild stress in rodents by increasing the mRNA levels of both GFAP and 5HT2B receptors in astrocytes (Dong et al. 2015).

Fluoxetine also increases the glio-transmission of ATP via vesicular nucleotide transporter (VNUT) transporter that results in activation of P2Y11 receptors and thereby increases BDNF in astrocytes, which promotes synaptic plasticity (Kinoshita et al. 2018). Given the commonality in all the above studies and the fact that an increase in BDNF levels produces anxiolytic and antidepressogenic-like effects in rodents, it becomes imperative that neuron-astroglia interplay is vital for the effectiveness of pharmacological interventions.

8.2 Atypical and Fast-Acting Antidepressants Modulate Astrocytic Activity

Unlike classical antidepressants, ketamine is a fast-acting antidepressant and is also effective in the treatment of drug-resistant depression. Ketamine modulates astrocytic cAMP signaling that plays important role in astrocyte arborization (Schiweck et al. 2018). Astrocytes are important for extracellular ion homeostasis. Ketamine is shown to directly regulate extracellular K^+ concentrations in lateral habenula of rodents in a study involving manipulation of surface availability of inward rectifying potassium channel (Kir4.1) on astrocyte (Cui et al. 2018).

Riluzole, a neuroprotective agent, used for the treatment of amyotrophic lateral sclerosis is also act as an antidepressant. It inhibits glutamate release in cultured neurons and is also shown to upregulate GLT-1 gene expression in cultured astrocytes. Increased expression of GLT-1 may be facilitating clearance of synaptic glutamate by astrocytes (Banasr et al. 2010). Besides pharmacological interventions, transcranial direct current-based interventions like electroconvulsive therapy is used in treatment of drug resistant subjects. A recent study has shown that electroconvulsive therapy increases GFAP expression and induces calcium signaling in astrocytes (Monai et al. 2016).

8.3 Epigenetic-Based Potential Antidepressive Molecules

Chromatin modifying agents and epigenetic-based potential therapeutic molecules have been demonstrated to modulate symptoms in depressive disorders. Most of these epigenetic-based antidepressants are nonspecific and affect both neurons and astrocytes. Valproic acid (VPA), a histone deacetylase inhibitor, acts as an antidepressant agent (Phiel et al. 2001). VPA treatment in astrocytes has been shown to increase H4Ac levels at GLT1 promoter which in turn promotes transcriptional expression of GLT1 (Perisic et al. 2012). The increased GLT1 level in astrocytes is envisioned to restore glutamine homeostasis. VPA treatment increases H3 acetylation and expression of neurotrophic factors like BDNF and GDNF (Wu et al. 2008). It has been proposed that treatments with antidepressants like amitriptyline, imipramine, and paroxetine ameliorate DNA methylation over the genes implicated in MDD, probably by reducing DNA methyl-transferase I activity in astrocytes (Zimmermann et al. 2012).

Chromatin modifying agents are being explored for reprogramming of reactive astrocytes into functional neurons in other neuropsychiatric disorders. Treatment with a mixture of chemical VPA, CHIR99021 and RepSox resulted in transcriptional induction of NeuroG2 and NeuroD1, which in turn reprograms astrocytes to neurons in the in vitro culture systems (Cheng et al. 2015). These results are promising and offer vast scope for manipulating resident astrocytes to restore neuronal deficits in the brain.

8.4 Genetic Manipulation of Astrocytes

Genetic manipulations of specific signaling pathways in astrocytes by gene knockout approaches are being studied to investigate astrocyte biology in detail. As mentioned earlier, several astrocyte signaling mechanisms have been implicated in mediating the normal functioning of the brain and are often perturbed in pathological conditions like depression. Overexpression of the mas-related gene A1 induces astrocytic Gq signaling, elevates intracellular Ca^{2+}, and ameliorates CSDS-induced depressive symptoms (Cao et al. 2013). Similarly, astrocyte-specific conditional GLT1 knockout mice exhibited abnormal repetitive behaviors that are relieved by systemic administration of an NMDA antagonist (Aida et al. 2015). Recently, a chemogenetic study involving hM3Dq DREADD-mediated enhanced activation of Gq signaling in the forebrain region during the postnatal period showed a long-lasting increase in anxiety, despair and schizophrenic-like behavior in adulthood. These behavioral changes were accompanied by altered glutamatergic and GABAergic metabolic activity in the cortex and hippocampus of the mice brain (Pati et al. 2020).

8.5 Exercise Modulates Astrocytic Function in Depressive Disorders

Physical activities are known to ameliorate depression-related symptoms. Among various physical activities, running has been demonstrated to have antidepressant effects in preclinical studies performed on the rodent model of depression (Li et al. 2021). Exercise-induced changes in astrocyte functions are being actively explored in normal and pathological conditions. An immunohistochemical study has reported increased numbers of astrocytes in the cingulate, visual, and motor cortex in rat performing treadmill exercise (Li et al. 2005). As mentioned earlier, depression results in astroglial atrophy in the hippocampus (Virmani et al. 2020), and running exercise has been shown to increase astrocyte numbers in the hippocampus in animal models of depression (Li et al. 2021).

8.6 Optogenetic-Based Modulation of Astrocytic Functions

Unlike the abovementioned approaches, optogenetics provides an opportunity for spatiotemporal manipulation of specific cell types in the brain. For optogenetic manipulation of astrocytes, light-sensitive protein, opsin, is expressed in an astrocyte-specific manner in the brain. Initially, Gourine's group applied optogenetic-based manipulation to astrocytes and demonstrated that light-mediated stimulation of channelrodopsin2 receptor (ChR2)-expressing astrocyte in the brain releases ATP along with changes in pH which regulates breathing (Gourine et al. 2010). Similar studies with light-stimulated ChR2-expressing astrocytes in the visual cortex demonstrated metabotropic glutamate receptor (mGluR1)-dependent enhanced synaptic transmission in neighboring neurons (Perea et al. 2014). More recently, manipulation of anterior cingulate with light stimulated channel rhodopsin was shown to regulate sleep-wake regulation (Yamashita et al. 2014). These studies demonstrated the manipulation of astrocytes activity in a region-specific manner could potentially augment neuronal activities. In a recent study, optogenetic stimulation of astrocytes in the basolateral amygdala (BLA) ameliorated anxiety in CUMS model of depression (Xiao et al. 2020).

9 Limitations

Astrocytes are understudied in comparison to neurons for several reasons. The limited understanding of astroglial biology, and its functions undermine efforts to target them for the purpose of effective treatment. In addressing astrocyte functions in the brain and psychiatric disorders, astrocytes were presumed to be homogenous. Recent research has established functional diversity in astrocytes and has shown the presence of functional subsets of astrocytes: five distinct astrocyte subpopulations with diverse cellular, molecular, and functional properties has been identified in adult brain (Lin et al. 2017). Only a specific perturbation of astrocytes subpopulation

is highly enriched in a disease condition. Hence, addressing heterogeneity in astrocytes has paramount importance in developing specific and effective treatment modalities. Otherwise targeting astrocytes in pathological conditions with a particular therapeutic agent may elicit an inadequate response or may override beneficial effects with unwanted side effects.

10 Conclusion

There has been a shift in the neuro-centric view of brain functions in appreciation of the role of other equally important brain cells. This paradigm shift approach has been instrumental in challenging previous understanding of the neuronal basis of behavior, and establishing/validating new theories in behavioral and molecular neurosciences. In this regard, astrocytes are emerging as prominent players in information processing and mediating behavioral outputs. Astrocytes being the primary elements of homeostatic regulatior in the brain, their perturbations appear to contribute to major depression and other neuropsychiatric disorders. Approaches to modulate astrocyte functions that include chemogenetics to more advanced optogenetics based approaches are continuously being developed. More studies are warranted to understand precise astroglia mechanisms in depressive disorders and to target astrocytes specifically for the development of novel therapeutics. The challenge of studying glial contribution to brain functions is further magnified by their heterogeneity. However, technological advancements in in vivo neuroimaging, multi-omics-based methods of establishing brain cell identity and phenotypes are envisioned to advance astroglia biology, and pinpointing astrocyte specific molecular targets for pharmacological intervention in depression. Overall, astrocytes are no longer mere glue or neuron-supporting cells in the brain but are emerging as the prime targets for the development of the next generation anti-depressants.

Acknowledgements This work was supported by funding from the Council for Scientific and Industrial Research, Government of India [Health Care Theme FBR/MLP0150] and Glia Initiative Project from the Department of Biotechnology, Government of India (BT/PR27426/MED/122/140/2018) to AK and ABP. AS thanks the Department of Biotechnology for the award of Junior Research fellowship.

References

Abdallah CG, Jiang L, De Feyter HM, Fasula M, Krystal JH, Rothman DL, Mason GF, Sanacora G (2014) Glutamate metabolism in major depressive disorder. Am J Psychiatry 171:1320–1327

Aida T, Yoshida J, Nomura M, Tanimura A, Iino Y, Soma M, Bai N, Ito Y, Cui W, Aizawa H, Yanagisawa M, Nagai T, Takata N, Tanaka KF, Takayanagi R, Kano M, Gotz M, Hirase H, Tanaka K (2015) Astroglial glutamate transporter deficiency increases synaptic excitability and leads to pathological repetitive behaviors in mice. Neuropsychopharmacology 40:1569–1579

Altshuler LL, Abulseoud OA, Foland-Ross L, Bartzokis G, Chang S, Mintz J, Hellemann G, Vinters HV (2010) Amygdala astrocyte reduction in subjects with major depressive disorder but not bipolar disorder. Bipolar Disord 12:541–549

Alvarez JI, Katayama T, Prat A (2013) Glial influence on the blood brain barrier. Glia 61:1939–1958

Arnone D, Mumuni AN, Jauhar S, Condon B, Cavanagh J (2015) Indirect evidence of selective glial involvement in glutamate-based mechanisms of mood regulation in depression: meta-analysis of absolute prefrontal neuro-metabolic concentrations. Eur Neuropsychopharmacol 25:1109–1117

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 21:1133–1145

Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S (2009) Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comparative Neurol 513(5):532–541

Bak LK, Schousboe A, Waagepetersen HS (2006) The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. J Neurochem 98:641–653

Banasr M, Chowdhury GM, Terwilliger R, Newton SS, Duman RS, Behar KL, Sanacora G (2010) Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. Mol Psychiatry 15:501–511

Benjamin AM, Quastel JH (1974) Fate of L-glutamate in the brain. J Neurochem 23:457–464

Bernard R, Kerman IA, Thompson RC, Jones EG, Bunney WE, Barchas JD, Schatzberg AF, Myers RM, Akil H, Watson SJ (2011) Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. Mol Psychiatry 16:634–646

Birch AM, Katsouri L, Sastre M (2014) Modulation of inflammation in transgenic models of Alzheimer's disease. J Neuroinflammation 11:1–13

Boyle MP, Brewer JA, Funatsu M, Wozniak DF, Tsien JZ, Izumi Y, Muglia LJ (2005) Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. Proc Natl Acad Sci U S A 102:473–478

Burda JE, Sofroniew MV (2014) Reactive gliosis and the multicellular response to CNS damage and disease. Neuron 81:229–248

Cao X, Li LP, Wang Q, Wu Q, Hu HH, Zhang M, Fang YY, Zhang J, Li SJ, Xiong WC, Yan HC, Gao YB, Liu JH, Li XW, Sun LR, Zeng YN, Zhu XH, Gao TM (2013) Astrocyte-derived ATP modulates depressive-like behaviors. Nat Med 19:773–777

Chandley MJ, Szebeni K, Szebeni A, Crawford J, Stockmeier CA, Turecki G, Miguel-Hidalgo JJ, Ordway GA (2013) Gene expression deficits in pontine locus coeruleus astrocytes in men with major depressive disorder. J Psychiatry Neurosci 38:276–284

Cheng L, Gao L, Guan W, Mao J, Hu W, Qiu B, Zhao J, Yu Y, Pei G (2015) Direct conversion of astrocytes into neuronal cells by drug cocktail. Cell Res 25:1269–1272

Cherix A, Larrieu T, Grosse J, Rodrigues J, McEwen B, Nasca C, Gruetter R, Sandi C (2020) Metabolic signature in nucleus accumbens for anti-depressant-like effects of acetyl-L-carnitine. elife 9:e50631

Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP, Myers RM, Bunney WE Jr, Akil H, Watson SJ, Jones EG (2005) Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. Proc Natl Acad Sci U S A 102:15653–15658

Chowdhury GM, Patel AB, Mason GF, Rothman DL, Behar KL (2007) Glutamatergic and GABAergic neurotransmitter cycling and energy metabolism in rat cerebral cortex during postnatal development. J Cereb Blood Flow Metab 27:1895–1907

Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA (2005) Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120:421–433

Chung WS, Allen NJ, Eroglu C (2015) Astrocytes control synapse formation, function, and elimination. Cold Spring Harb Perspect Biol 7:a020370

Cobb JA, O'Neill K, Milner J, Mahajan GJ, Lawrence TJ, May WL, Miguel-Hidalgo J, Rajkowska G, Stockmeier CA (2016) Density of GFAP-immunoreactive astrocytes is decreased in left hippocampi in major depressive disorder. Neuroscience 316:209–220

Colpo GD, Venna VR, McCullough LD, Teixeira AL (2019) Systematic review on the involvement of the kynurenine pathway in stroke: pre-clinical and clinical evidence. Front Neurol 10:778

Connor TJ, Starr N, O'Sullivan JB, Harkin A (2008) Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma? Neurosci Lett 441:29–34

Cotter D, Mackay D, Landau S, Kerwin R, Everall I (2001) Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. Arch Gen Psychiatry 58:545–553

Cui Y, Yang Y, Ni Z, Dong Y, Cai G, Foncelle A, Ma S, Sang K, Tang S, Li Y (2018) Astroglial Kir4. 1 in the lateral habenula drives neuronal bursts in depression. Nature 554:323–327

Czeh B, Nagy SA (2018) Clinical findings documenting cellular and molecular abnormalities of glia in depressive disorders. Front Mol Neurosci 11:56

de Diego-Adelino J, Portella MJ, Gomez-Anson B, Lopez-Moruelo O, Serra-Blasco M, Vives Y, Puigdemont D, Perez-Egea R, Alvarez E, Perez V (2013) Hippocampal abnormalities of glutamate/glutamine, N-acetylaspartate and choline in patients with depression are related to past illness burden. J Psychiatry Neurosci 38:107–116

Dedovic K, Duchesne A, Andrews J, Engert V, Pruessner JC (2009) The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. NeuroImage 47:864–871

Dietz AG, Goldman SA, Nedergaard M (2020) Glial cells in schizophrenia: a unified hypothesis. Lancet Psychiatry 7:272–281

Dong L, Li B, Verkhratsky A, Peng L (2015) Cell type-specific *in vivo* expression of genes encoding signalling molecules in the brain in response to chronic mild stress and chronic treatment with fluoxetine. Psychopharmacology 232:2827–2835

Duman RS, Aghajanian GK, Sanacora G, Krystal JH (2016) Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat Med 22:238–249

Duman RS, Sanacora G, Krystal JH (2019) Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversal by novel treatments. Neuron 102:75–90

Dupont AC, Largeau B, Santiago Ribeiro MJ, Guilloteau D, Tronel C, Arlicot N (2017) Translocator Protein-18 kDa (TSPO) positron emission tomography (PET) imaging and its clinical impact in neurodegenerative diseases. Int J Mol Sci 18:785

Edwards RH (2007) The neurotransmitter cycle and quantal size. Neuron 55:835–858

Elbejjani M, Fuhrer R, Abrahamowicz M, Mazoyer B, Crivello F, Tzourio C, Dufouil C (2015) Depression, depressive symptoms, and rate of hippocampal atrophy in a longitudinal cohort of older men and women. Psychol Med 45:1931–1944

Enache D, Pariante CM, Mondelli V (2019) Markers of central inflammation in major depressive disorder: a systematic review and meta-analysis of studies examining cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. Brain Behav Immun 81:24–40

Fatemi SH, Laurence JA, Araghi-Niknam M, Stary JM, Schulz SC, Lee S, Gottesman II (2004) Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia. Schizophr Res 69:317–323

Felger JC, Haroon E, Patel TA, Goldsmith DR, Wommack EC, Woolwine BJ, Le NA, Feinberg R, Tansey MG, Miller AH (2020) What does plasma CRP tell us about peripheral and central inflammation in depression? Mol Psychiatry 25:1301–1311

Fields RD, Araque A, Johansen-Berg H, Lim SS, Lynch G, Nave KA, Nedergaard M, Perez R, Sejnowski T, Wake H (2014) Glial biology in learning and cognition. Neuroscientist 20:426–431

Filosa JA, Morrison HW, Iddings JA, Du W, Kim KJ (2016) Beyond neurovascular coupling, role of astrocytes in the regulation of vascular tone. Neuroscience 323:96–109

Gao Y, Ma J, Tang J, Liang X, Huang CX, Wang SR, Chen LM, Wang FF, Tan CX, Chao FL, Zhang L, Qiu X, Luo YM, Xiao Q, Du L, Xiao Q, Tang Y (2017) White matter atrophy and myelinated fiber disruption in a rat model of depression. J Comp Neurol 525:1922–1933

GBD 2016 Disease and Injury Incidence and Prevalence Collaborators (2018) Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392:1789–1858

Gittins RA, Harrison PJ (2011) A morphometric study of glia and neurons in the anterior cingulate cortex in mood disorder. J Affect Disord 133:328–332

Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, Teschemacher AG, Spyer KM, Deisseroth K, Kasparov S (2010) Astrocytes control breathing through pH-dependent release of ATP. Science 329:571–575

Gruetter R, Adriany G, Choi IY, Henry PG, Lei H, Oz G (2003) Localized *in vivo* ^{13}C NMR spectroscopy of the brain. NMR Biomed 16:313–338

Hannestad J, DellaGioia N, Gallezot JD, Lim K, Nabulsi N, Esterlis I, Pittman B, Lee JY, O'Connor KC, Pelletier D, Carson RE (2013) The neuroinflammation marker translocator protein is not elevated in individuals with mild-to-moderate depression: a [(1)(1)C]PBR28 PET study. Brain Behav Immun 33:131–138

Harmer CJ, Duman RS, Cowen PJ (2017) How do antidepressants work? New perspectives for refining future treatment approaches. Lancet Psychiatry 4:409–418

Hertz L, Dringen R, Schousboe A, Robinson SR (1999) Astrocytes: glutamate producers for neurons. J Neurosci Res 57:417–428

Hertz L, Li B, Song D, Ren J, Dong L, Chen Y, Peng L (2012) Astrocytes as a 5-HT2B-mediated SERT-independent SSRI target, slowly altering depression-associated genes and function. Curr Signal Transduct Ther 7:65–80

Hertz L, Rothman DL, Li B, Peng L (2015) Chronic SSRI stimulation of astrocytic 5-HT2B receptors change multiple gene expressions/editings and metabolism of glutamate, glucose and glycogen: a potential paradigm shift. Front Behav Neurosci 9:25

Holmes SE, Hinz R, Conen S, Gregory CJ, Matthews JC, Anton-Rodriguez JM, Gerhard A, Talbot PS (2018) Elevated translocator protein in anterior cingulate in major depression and a role for inflammation in suicidal thinking: a positron emission tomography study. Biol Psychiatry 83: 61–69

Holsboer F (2000) The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23:477–501

Howard DM, Adams MJ, Clarke TK, Hafferty JD, Gibson J, Shirali M, Coleman JRI, Hagenaars SP, Ward J, Wigmore EM, Alloza C, Shen X, Barbu MC, Xu EY, Whalley HC, Marioni RE, Porteous DJ, Davies G, Deary IJ, Hemani G, Berger K, Teismann H, Rawal R, Arolt V, Baune BT, Dannlowski U, Domschke K, Tian C, Hinds DA, 23andMe Research Team, Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium, Trzaskowski M, Byrne EM, Ripke S, Smith DJ, Sullivan PF, Wray NR, Breen G, Lewis CM, McIntosh AM (2019) Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. Nat Neurosci 22:343–352

Howarth C, Gleeson P, Attwell D (2012) Updated energy budgets for neural computation in the neocortex and cerebellum. J Cereb Blood Flow Metab 32:1222–1232

Huang D, Li C, Zhang W, Qin J, Jiang W, Hu C (2019) Dysfunction of astrocytic connexins 30 and 43 in the medial prefrontal cortex and hippocampus mediates depressive-like behaviours. Behav Brain Res 372:111950

Hyder F, Patel AB, Gjedde A, Rothman DL, Behar KL, Shulman RG (2006) Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. J Cereb Blood Flow Metab 26:865–877

Jarnum H, Eskildsen SF, Steffensen EG, Lundbye-Christensen S, Simonsen CW, Thomsen IS, Frund ET, Theberge J, Larsson EM (2011) Longitudinal MRI study of cortical thickness, perfusion, and metabolite levels in major depressive disorder. Acta Psychiatr Scand 124:435–446

Johnston-Wilson NL, Sims CD, Hofmann JP, Anderson L, Shore AD, Torrey EF, Yolken RH (2000) Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder. The Stanley Neuropathology Consortium. Mol Psychiatry 5:142–149

Jollant F, Near J, Turecki G, Richard-Devantoy S (2016) Spectroscopy markers of suicidal risk and mental pain in depressed patients. Prog Neuropsychopharmacol Biol Psychiatry. https://doi.org/10.1016/j.pnpbp.2016.10.005

Juruena MF, Bocharova M, Agustini B, Young AH (2018) Atypical depression and non-atypical depression: is HPA axis function a biomarker? A systematic review. J Affect Disord 233:45–67

Kahl KG, Atalay S, Maudsley AA, Sheriff S, Cummings A, Frieling H, Schmitz B, Lanfermann H, Ding XQ (2020) Altered neurometabolism in major depressive disorder: a whole brain (1)H-magnetic resonance spectroscopic imaging study at 3T. Prog Neuropsychopharmacol Biol Psychiatry 101:109916

Kempton MJ, Salvador Z, Munafo MR, Geddes JR, Simmons A, Frangou S, Williams SC (2011) Structural neuroimaging studies in major depressive disorder. Meta-analysis and comparison with bipolar disorder. Arch Gen Psychiatry 68:675–690

Keshavarz M (2017) Glial cells as key elements in the pathophysiology and treatment of bipolar disorder. Acta Neuropsychiatr 29:140–152

Kim R, Healey KL, Sepulveda-Orengo MT, Reissner KJ (2018) Astroglial correlates of neuropsychiatric disease: from astrocytopathy to astrogliosis. Prog Neuropsychopharmacol Biol Psychiatry 87:126–146

Kinoshita M, Hirayama Y, Fujishita K, Shibata K, Shinozaki Y, Shigetomi E, Takeda A, Le HPN, Hayashi H, Hiasa M, Moriyama Y, Ikenaka K, Tanaka KF, Koizumi S (2018) Anti-depressant fluoxetine reveals its therapeutic effect via astrocytes. EBioMedicine 32:72–83

Kohler S, Winkler U, Hirrlinger J (2021) Heterogeneity of astrocytes in grey and white matter. Neurochem Res 46:3–14

Laugeray A, Launay JM, Callebert J, Mutlu O, Guillemin GJ, Belzung C, Barone PR (2016) Chronic treatment with the IDO1 inhibitor 1-methyl-D-tryptophan minimizes the behavioural and biochemical abnormalities induced by unpredictable chronic mild stress in mice - comparison with fluoxetine. PLoS One 11:e0164337

Lebon V, Petersen KF, Cline GW, Shen J, Mason GF, Dufour S, Behar KL, Shulman GI, Rothman DL (2002) Astroglial contribution to brain energy metabolism in humans revealed by ^{13}C nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism. J Neurosci 22:1523–1531

Lehmann ML, Weigel TK, Elkahloun AG, Herkenham M (2017) Chronic social defeat reduces myelination in the mouse medial prefrontal cortex. Sci Rep 7:46548

Lener MS, Niciu MJ, Ballard ED, Park M, Park LT, Nugent AC, Zarate CA Jr (2017) Glutamate and gamma-aminobutyric acid systems in the pathophysiology of major depression and antidepressant response to ketamine. Biol Psychiatry 81:886–897

Lewis CP, Port JD, Blacker CJ, Sonmez AI, Seewoo BJ, Leffler JM, Frye MA, Croarkin PE (2020) Altered anterior cingulate glutamatergic metabolism in depressed adolescents with current suicidal ideation. Transl Psychiatry 10:119

Li J, Ding YH, Rafols JA, Lai Q, McAllister JP II, Ding Y (2005) Increased astrocyte proliferation in rats after running exercise. Neurosci Lett 386:160–164

Li B, Zhang S, Li M, Hertz L, Peng L (2009) Chronic treatment of astrocytes with therapeutically relevant fluoxetine concentrations enhances cPLA2 expression secondary to 5-HT2B-induced, transactivation-mediated ERK1/2 phosphorylation. Psychopharmacology 207:1–12

Li H, Sagar AP, Keri S (2018) Translocator protein (18kDa TSPO) binding, a marker of microglia, is reduced in major depression during cognitive-behavioral therapy. Prog Neuropsychopharmacol Biol Psychiatry 83:1–7

Li Y, Luo Y, Tang J, Liang X, Wang J, Xiao Q, Zhu P, Xiao K, Jiang L, Dou X (2021) The positive effects of running exercise on hippocampal astrocytes in a rat model of depression. Transl Psychiatry 11:1–13

Lin C-CJ, Yu K, Hatcher A, Huang T-W, Lee HK, Carlson J, Weston MC, Chen F, Zhang Y, Zhu W (2017) Identification of diverse astrocyte populations and their malignant analogs. Nat Neurosci 20:396–405

Luykx JJ, Laban KG, van den Heuvel MP, Boks MP, Mandl RC, Kahn RS, Bakker SC (2012) Region and state specific glutamate downregulation in major depressive disorder: a meta-analysis of (1)H-MRS findings. Neurosci Biobehav Rev 36:198–205

Magistretti PJ, Pellerin L, Rothman DL, Shulman RG (1999) Energy on demand. Science 283:496–497

Mason GF, Gruetter R, Rothman DL, Behar KL, Shulman RG, Novotny EJ (1995) Simultaneous determination of the rates of the TCA cycle, glucose utilization, alpha-ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. J Cereb Blood Flow Metab 15:12–25

Meyer JH, Cervenka S, Kim MJ, Kreisl WC, Henter ID, Innis RB (2020) Neuroinflammation in psychiatric disorders: PET imaging and promising new targets. Lancet Psychiatry 7:1064–1074

Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G (2000) Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. Biol Psychiatry 48:861–873

Miguel-Hidalgo JJ, Waltzer R, Whittom AA, Austin MC, Rajkowska G, Stockmeier CA (2010) Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. J Affect Disord 127:230–240

Miguel-Hidalgo JJ, Wilson BA, Hussain S, Meshram A, Rajkowska G, Stockmeier CA (2014) Reduced connexin 43 immunolabeling in the orbitofrontal cortex in alcohol dependence and depression. J Psychiatr Res 55:101–109

Miguel-Hidalgo JJ, Moulana M, Deloach PH, Rajkowska G (2018) Chronic unpredictable stress reduces immunostaining for Connexins 43 and 30 and myelin basic protein in the rat prelimbic and orbitofrontal cortices. Chronic Stress (Thousand Oaks) 2:2470547018814186

Mishra PK, Kumar A, Behar KL, Patel AB (2018) Subanesthetic ketamine reverses neuronal and astroglial metabolic activity deficits in a social defeat model of depression. J Neurochem 146:722–734

Mishra PK, Adusumilli M, Deolal P, Mason GF, Kumar A, Patel AB (2020) Impaired neuronal and astroglial metabolic activity in chronic unpredictable mild stress model of depression: reversal of behavioral and metabolic deficit with lanicemine. Neurochem Int 137:104750

Monai H, Ohkura M, Tanaka M, Oe Y, Konno A, Hirai H, Mikoshiba K, Itohara S, Nakai J, Iwai Y, Hirase H (2016) Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. Nat Commun 7:11100

Mondal AC, Fatima M (2019) Direct and indirect evidences of BDNF and NGF as key modulators in depression: role of antidepressants treatment. Int J Neurosci 129:283–296

Muller MB, Lucassen PJ, Yassouridis A, Hoogendijk WJ, Holsboer F, Swaab DF (2001) Neither major depression nor glucocorticoid treatment affects the cellular integrity of the human hippocampus. Eur J Neurosci 14:1603–1612

Nagy C, Torres-Platas SG, Mechawar N, Turecki G (2017) Repression of astrocytic connexins in cortical and subcortical brain regions and prefrontal enrichment of H3K9me3 in depression and suicide. Int J Neuropsychopharmacol 20:50–57

Nair A, Bonneau RH (2006) Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. J Neuroimmunol 171:72–85

Nave KA (2010) Myelination and support of axonal integrity by glia. Nature 468:244–252

O'Leary LA, Mechawar N (2021) Implication of cerebral astrocytes in major depression: a review of fine neuroanatomical evidence in humans. Glia 69(9):2077–2099

O'Leary LA, Belliveau C, Davoli MA, Ma JC, Tanti A, Turecki G, Mechawar N (2021) Widespread decrease of cerebral vimentin-immunoreactive astrocytes in depressed suicides. Front Psychiatry 12:640963

Owen DR, Narayan N, Wells L, Healy L, Smyth E, Rabiner EA, Galloway D, Williams JB, Lehr J, Mandhair H, Peferoen LA, Taylor PC, Amor S, Antel JP, Matthews PM, Moore CS (2017) Pro-inflammatory activation of primary microglia and macrophages increases 18 kDa translocator protein expression in rodents but not humans. J Cereb Blood Flow Metab 37:2679–2690

Park C, Rosenblat JD, Brietzke E, Pan Z, Lee Y, Cao B, Zuckerman H, Kalantarova A, McIntyre RS (2019) Stress, epigenetics and depression: a systematic review. Neurosci Biobehav Rev 102:139–152

Parrott JM, Redus L, Santana-Coelho D, Morales J, Gao X, O'Connor JC (2016) Neurotoxic kynurenine metabolism is increased in the dorsal hippocampus and drives distinct depressive behaviors during inflammation. Transl Psychiatry 6:e918

Paslakis G, Traber F, Roberz J, Block W, Jessen F (2014) N-acetyl-aspartate (NAA) as a correlate of pharmacological treatment in psychiatric disorders: a systematic review. Eur Neuropsychopharmacol 24:1659–1675

Patel AB, Rothman DL, Cline GW, Behar KL (2001) Glutamine is the major precursor for GABA synthesis in rat neocortex *in vivo* following acute GABA-transaminase inhibition. Brain Res 919:207–220

Patel AB, de Graaf RA, Mason GF, Kanamatsu T, Rothman DL, Shulman RG, Behar KL (2004) Glutamatergic neurotransmission and neuronal glucose oxidation are coupled during intense neuronal activation. J Cereb Blood Flow Metab 24:972–985

Patel AB, de Graaf RA, Mason GF, Rothman DL, Shulman RG, Behar KL (2005) The contribution of GABA to glutamate/glutamine cycling and energy metabolism in the rat cortex *in vivo*. Proc Natl Acad Sci U S A 102:5588–5593

Patel AB, de Graaf RA, Rothman DL, Behar KL, Mason GF (2010) Evaluation of cerebral acetate transport and metabolic rates in the rat brain *in vivo* using ^{1}H-[^{13}C]-NMR. J Cereb Blood Flow Metab 30:1200–1213

Patel AB, Tiwari V, Veeraiah P, Saba K (2018) Increased astroglial activity and reduced neuronal function across brain in AbetaPP-PS1 mouse model of Alzheimer's disease. J Cereb Blood Flow Metab 38:1213–1226

Pati S, Saba K, Salvi SS, Tiwari P, Chaudhari PR, Verma V, Mukhopadhyay S, Kapri D, Suryavanshi S, Clement JP, Patel AB, Vaidya VA (2020) Chronic postnatal chemogenetic activation of forebrain excitatory neurons evokes persistent changes in mood behavior. Elife 9: e56171

Pellerin L, Pellegri G, Bittar PG, Charnay Y, Bouras C, Martin JL, Stella N, Magistretti PJ (1998) Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. Dev Neurosci 20:291–299

Perea G, Yang A, Boyden ES, Sur M (2014) Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons *in vivo*. Nat Commun 5:1–12

Perez-Caballero L, Torres-Sanchez S, Romero-Lopez-Alberca C, Gonzalez-Saiz F, Mico JA, Berrocoso E (2019) Monoaminergic system and depression. Cell Tissue Res 377:107–113

Perisic T, Holsboer F, Rein T, Zschocke J (2012) The CpG island shore of the GLT-1 gene acts as a methylation-sensitive enhancer. Glia 60:1345–1355

Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS (2001) Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J Biol Chem 276:36734–36741

Rajkowska G, Miguel-Hidalgo JJ (2007) Gliogenesis and glial pathology in depression. CNS Neurol Disord Drug Targets 6:219–233

Rajkowska G, Hughes J, Stockmeier CA, Javier Miguel-Hidalgo J, Maciag D (2013) Coverage of blood vessels by astrocytic endfeet is reduced in major depressive disorder. Biol Psychiatry 73: 613–621

Rajkowska G, Legutko B, Moulana M, Syed M, Romero DG, Stockmeier CA, Miguel-Hidalgo JJ (2018) Astrocyte pathology in the ventral prefrontal white matter in depression. J Psychiatr Res 102:150–158

Richards EM, Zanotti-Fregonara P, Fujita M, Newman L, Farmer C, Ballard ED, Machado-Vieira-R, Yuan P, Niciu MJ, Lyoo CH, Henter ID, Salvadore G, Drevets WC, Kolb H, Innis RB, Zarate CA Jr (2018) PET radioligand binding to translocator protein (TSPO) is increased in unmedicated depressed subjects. EJNMMI Res 8:57

Rivera AD, Butt AM (2019) Astrocytes are direct cellular targets of lithium treatment: novel roles for lysyl oxidase and peroxisome-proliferator activated receptor-gamma as astroglial targets of lithium. Transl Psychiatry 9:211

Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N, Kuncl RW (1994) Localization of neuronal and glial glutamate transporters. Neuron 13:713–725

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16:675–686

Saito T, Tamura M, Chiba Y, Katsuse O, Suda A, Kamada A, Ikura T, Abe K, Ogawa M, Minegishi K, Yoshimi R, Kirino Y, Ihata A, Hirayasu Y (2017) Regional cerebral glucose metabolism in systemic lupus erythematosus patients with major depressive disorder. J Neurol Sci 379:127–130

Sarawagi A, Soni ND, Patel AB (2021) Glutamate and GABA homeostasis and neurometabolism in major depressive disorder. Front Psychiatry 12:637863

Schiweck J, Eickholt BJ, Murk K (2018) Important shapeshifter: mechanisms allowing astrocytes to respond to the changing nervous system during development, injury and disease. Front Cell Neurosci 12:261

Schmaal L, Pozzi E, Ho TC, van Velzen LS, Veer IM, Opel N, Van Someren EJW, Han LKM, Aftanas L, Aleman A, Baune BT, Berger K, Blanken TF, Capitao L, Couvy-Duchesne B, Cullen KR, Dannlowski U, Davey C, Erwin-Grabner T, Evans J, Frodl T, Fu CHY, Godlewska B, Gotlib IH, Goya-Maldonado R, Grabe HJ, Groenewold NA, Grotegerd D, Gruber O, Gutman BA, Hall GB, Harrison BJ, Hatton SN, Hermesdorf M, Hickie IB, Hilland E, Irungu B, Jonassen R, Kelly S, Kircher T, Klimes-Dougan B, Krug A, Landro NI, Lagopoulos J, Leerssen J, Li M, Linden DEJ, MacMaster FP, McIntosh AM, Mehler DMA, Nenadic I, Penninx B, Portella MJ, Reneman L, Renteria ME, Sacchet MD, Samann PG, Schrantee A, Sim K, Soares JC, Stein DJ, Tozzi L, van der Wee NJA, van Tol MJ, Vermeiren R, Vives-Gilabert Y, Walter H, Walter M, Whalley HC, Wittfeld K, Whittle S, Wright MJ, Yang TT, Zarate C Jr, Thomopoulos SI, Jahanshad N, Thompson PM, Veltman DJ (2020) ENIGMA MDD: seven years of global neuroimaging studies of major depression through worldwide data sharing. Transl Psychiatry 10:172

Sequeira A, Mamdani F, Ernst C, Vawter MP, Bunney WE, Lebel V, Rehal S, Klempan T, Gratton A, Benkelfat C, Rouleau GA, Mechawar N, Turecki G (2009) Global brain gene expression analysis links glutamatergic and GABAergic alterations to suicide and major depression. PLoS One 4:e6585

Setiawan E, Attwells S, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Xu C, Sharma S, Kish S, Houle S, Meyer JH (2018) Association of translocator protein total distribution volume with duration of untreated major depressive disorder: a cross-sectional study. Lancet Psychiatry 5: 339–347

Shen J, Petersen KF, Behar KL, Brown P, Nixon TW, Mason GF, Petroff OA, Shulman GI, Shulman RG, Rothman DL (1999) Determination of the rate of the glutamate/glutamine cycle in the human brain by *in vivo* ^{13}C NMR. Proc Natl Acad Sci U S A 96:8235–8240

Si X, Miguel-Hidalgo JJ, O'Dwyer G, Stockmeier CA, Rajkowska G (2004) Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. Neuropsychopharmacology 29:2088–2096

Sibson NR, Dhankhar A, Mason GF, Rothman DL, Behar KL, Shulman RG (1998) Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. Proc Natl Acad Sci U S A 95:316–321

Simard M, Nedergaard M (2004) The neurobiology of glia in the context of water and ion homeostasis. Neuroscience 129:877–896

Soghomonian JJ, Martin DL (1998) Two isoforms of glutamate decarboxylase: why? Trends Pharmacol Sci 19:500–505

Sokoloff L (1977) Relation between physiological function and energy metabolism in the central nervous system. J Neurochem 29:13–26

Soni ND, Ramesh A, Roy D, Patel AB (2021) Brain energy metabolism in intracerebroventricularly administered streptozotocin mouse model of Alzheimer's disease: a (1)H-[(13)C]-NMR study. J Cereb Blood Flow Metab 2021:271678X21996176

Sun JD, Liu Y, Yuan YH, Li J, Chen NH (2012) Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. Neuropsychopharmacology 37:1305–1320

Tiwari V, Ambadipudi S, Patel AB (2013) Glutamatergic and GABAergic TCA cycle and neurotransmitter cycling fluxes in different regions of mouse brain. J Cereb Blood Flow Metab 33: 1523–1531

Troubat R, Barone P, Leman S, Desmidt T, Cressant A, Atanasova B, Brizard B, El Hage W, Surget A, Belzung C, Camus V (2021) Neuroinflammation and depression: a review. Eur J Neurosci 53:151–171

Ullian EM, Sapperstein SK, Christopherson KS, Barres BA (2001) Control of synapse number by glia. Science 291:657–661

Uranova NA, Vostrikov VM, Orlovskaya DD, Rachmanova VI (2004) Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium. Schizophr Res 67:269–275

Veeraiah P, Noronha JM, Maitra S, Bagga P, Khandelwal N, Chakravarty S, Kumar A, Patel AB (2014) Dysfunctional glutamatergic and gamma-aminobutyric acidergic activities in prefrontal cortex of mice in social defeat model of depression. Biol Psychiatry 76:231–238

Virmani G, D'Almeida P, Nandi A, Marathe S (2020) Subfield-specific effects of chronic mild unpredictable stress on hippocampal astrocytes. bioRxiv

Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 6:626–640

World Health Organization (2017) Depression and other common mental disorders: global health estimates. World Health Organization, Geneva

Wu X, Chen PS, Dallas S, Wilson B, Block ML, Wang CC, Kinyamu H, Lu N, Gao X, Leng Y, Chuang DM, Zhang W, Lu RB, Hong JS (2008) Histone deacetylase inhibitors up-regulate astrocyte GDNF and BDNF gene transcription and protect dopaminergic neurons. Int J Neuropsychopharmacol 11:1123–1134

Xiao Q, Xu X, Tu J (2020) Chronic optogenetic manipulation of basolateral amygdala astrocytes rescues stress-induced anxiety. Biochem Biophys Res Commun 533:657–664

Xin L, Mlynarik V, Lanz B, Frenkel H, Gruetter R (2010) ^{1}H-[^{13}C] NMR spectroscopy of the rat brain during infusion of [2-13C] acetate at 14.1 T. Magn Reson Med 64:334–340

Yamashita A, Hamada A, Suhara Y, Kawabe R, Yanase M, Kuzumaki N, Narita M, Matsui R, Okano H, Narita M (2014) Astrocytic activation in the anterior cingulate cortex is critical for sleep disorder under neuropathic pain. Synapse 68:235–247

Yuan Y-M, He C (2013) The glial scar in spinal cord injury and repair. Neurosci Bull 29:421–435

Zhang JC, Yao W, Hashimoto K (2016) Brain-derived neurotrophic factor (BDNF)-TrkB signaling in inflammation-related depression and potential therapeutic targets. Curr Neuropharmacol 14: 721–731

Zhou B, Zhu Z, Ransom BR, Tong X (2021) Oligodendrocyte lineage cells and depression. Mol Psychiatry 26:103–117

Zhu X, Ye G, Wang Z, Luo J, Hao X (2017) Sub-anesthetic doses of ketamine exert antidepressant-like effects and upregulate the expression of glutamate transporters in the hippocampus of rats. Neurosci Lett 639:132–137

Zimmermann N, Zschocke J, Perisic T, Yu S, Holsboer F, Rein T (2012) Antidepressants inhibit DNA methyltransferase 1 through reducing G9a levels. Biochem J 448:93–102

Zink M, Vollmayr B, Gebicke-Haerter PJ, Henn FA (2010) Reduced expression of glutamate transporters vGluT1, EAAT2 and EAAT4 in learned helpless rats, an animal model of depression. Neuropharmacology 58:465–473

Zong H, Parada LF, Baker SJ (2015) Cell of origin for malignant gliomas and its implication in therapeutic development. Cold Spring Harb Perspect Biol 7:a020610

Glia in Epilepsy: An Overview

Aastha Dheer, Aparna Banerjee Dixit, Manjari Tripathi, P. Sarat Chandra, and Jyotirmoy Banerjee

Abstract

Epilepsy is one of the most common neurological disorders affecting 1% of the world population and is characterized by recurrent, spontaneous seizures caused due to hyperexcitability and hypersynchrony of neurons. Nearly one-third of the patients having seizures are drug resistant that is they do not respond to antiepileptic drugs (AEDs); therefore, a deeper understanding of the underlying mechanisms is required to develop more effective therapies. Most of the AEDs target neuronal mechanisms, however, the glia outnumbers the neurons in the CNS and are involved in controlling diverse neuronal functions. The understanding of the role of glia in epilepsy is therefore pertinent for knowing the pathophysiology of epilepsy. Altered glial functions may promote epileptogenesis. Astrocyte and microglia activation, gliosis, and glial tumors are reported to be associated with the epilepsy. Astrocytes regulate water and K^+ flow providing osmotic spatial buffering. The release of gliotransmitters like glutamate, adenosine, and ATP plays important roles in the pathophysiology. Astrocytes are intimately related to the blood vessels and regulate the blood-brain barrier functions by releasing chemical signals that maintain tight junction formation and their function. The brain microvasculature undergoes several alterations in

A. Dheer · P. S. Chandra
Department of Neurosurgery, All India Institute of Medical Sciences (AIIMS), New Delhi, India

A. B. Dixit
Dr. B. R. Ambedkar Centre for Biomedical Research, University of Delhi, New Delhi, India

M. Tripathi
Department of Neurology, All India Institute of Medical Sciences (AIIMS), New Delhi, India

J. Banerjee (✉)
Department of Biophysics, All India Institute of Medical Sciences (AIIMS), New Delhi, India
e-mail: jyotirmoy@aiims.edu

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_12

epilepsy. Astrocytes are coupled with different cells via gap junctions (GJs) which provide cellular communication, regulate K^+ and glutamate redistribution, and mediate synapse function and memory formation. Microglia, the innate immune cells of the CNS have crucial role in both physiological and pathological conditions. Glia-mediated inflammation has been known to promote epileptogenesis. The release of chemokines, pro-inflammatory cytokines like IL-β1, TNF-α, and many others, is reported to be associated with seizure frequency and disease duration in various epilepsy pathologies like mesial temporal sclerosis, focal cortical dysplasia, and Rasmussen's encephalitis. Thus, the interplay between microglia and astrocytes is crucial in epileptogenesis. In this chapter, we aim to provide an overview of the current understanding of the role of astrocytes and microglia in the pathophysiology of epilepsy discussing their function in ion homeostasis, glutamate metabolism, gliotransmission, maintenance of the blood-brain barrier, and inflammation.

Keywords

Epilepsy · Epileptogenesis · Astrogliosis · Microgliosis · Antiepileptic drugs

1 Brief Introduction to Epilepsy

Epilepsy is one of the most widespread neurologic disorders, affecting many individuals during their lifetime. The International League against Epilepsy (ILAE) defined epilepsy as the condition of minimum two unprovoked seizures occurring more than 24 h apart or one unprovoked seizure with a possibility of reappearance of more seizures after two unprovoked seizures, occurring over the next 10 years or if there is diagnosis of an epilepsy syndrome (Fisher et al. 2014). The clinical manifestations of epilepsy are multifaceted and heterogeneous which could be just a subtle interruption of awareness and responsiveness or an out of proportion convulsion of the entire body. The ILAE uses several levels to classify epilepsy to provide better diagnosis and understanding of the pathology. The three major levels used for classification of epilepsy are the seizure type, epilepsy type, and epilepsy syndrome following which epilepsies are classified as generalized epilepsy, focal epilepsy, combined generalized and focal epilepsy, and unknown epilepsy (Fisher et al. 2017). A "seizure" may be defined as an abnormal excessive or synchronous neuronal activity in the brain. The causes of seizures are extremely varied like acquired (symptomatic) as may be observed in meningitis, traumatic brain injury, and hypoxia-ischemia. They can also be metabolic, congenital, genetic, or idiopathic (Pack 2019). The new classification of seizures by ILAE was released in 2017 and provides a better, comprehensive, and detailed understanding of the seizure types making diagnosis easier. The basic guidelines for seizure classification used are site of onset of seizure, level of awareness, and involvement of other symptoms like movement (Fisher et al. 2017). Seizures are primarily classified based on the initial appearances of the seizure based on which they may be focal,

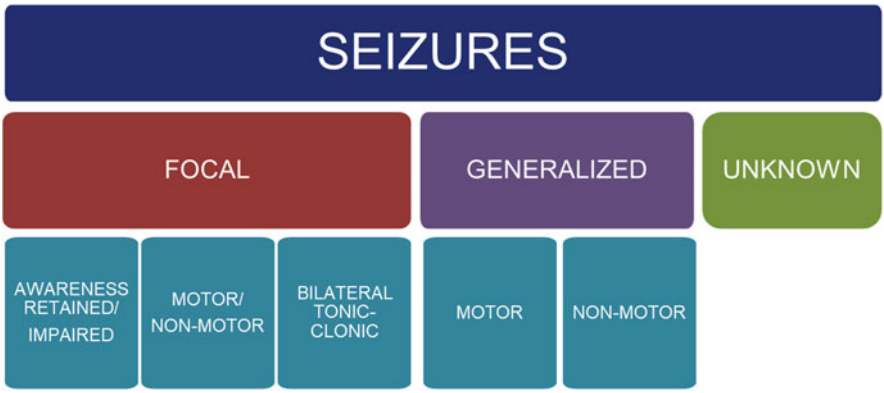

Fig. 1 Seizure type classification. (Adapted from "Basic version of 2017 International League Against Epilepsy Seizure Type Classification" (Fisher et al. 2017; Sarmast et al. 2020))

i.e., seizures originate within a discretely localized or widely distributed neuronal network limited to one hemisphere or generalized seizures which originate at a point and rapidly involve bilateral distributed neural networks. The seizures are classified as unknown if the seizure onset is not clear or missed. Focal seizures are subdivided as (a) awareness retained or impaired, (b) motor or non-motor, and (c) focal or bilateral tonic-clonic. Generalized seizures may be motor or non-motor (absence) seizures (Fisher et al. 2017; Sarmast et al. 2020) (Fig. 1).

Epilepsy is a complex pathology and requires a great deal of understanding for developing better therapeutic interventions. The medical treatment may include administering of antiepileptic drugs (AEDs) and dietary or lifestyle changes (like ketogenic diet) and in cases there may be a need for surgical treatment (Stafstrom and Carmant 2015). Even with the advancements in antiepileptic therapy, nearly 25% of epilepsy patients so not respond to medication. The International League Against Epilepsy (ILAE) defines drug-resistant epilepsy (DRE) as "failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" (Kwan et al. 2010). About one-third of all patients with epilepsy manifest drug-resistant epilepsy (Shorvon and Goodridge 2013). Some patients respond to AED, nearly 4% of adult cases yearly and higher rate in children, but this is temporary and seizures often reappear (Ramos-Lizana et al. 2009). Therefore, the only option that remains is surgery for patients with drug refractory epilepsies (Sheng et al. 2018).

Pathogenesis of DRE is explained by the following popular theories which are (a) transporter hypothesis, (b) target hypothesis, and (c) a recently proposed neural network hypothesis. The transporter hypothesis proposes that due to altered expression of multidrug transporters present in the blood-brain barrier which are crucial for the efflux of drugs or vesicles from the endothelial cells comprising the BBB, they are critical in causing decreased intracellular drug concentrations or distribution leading to drug resistance (Löscher et al. 2011; Löscher and Potschka 2005;

Margineanu and Klitgaard 2009). Molecules like P-glycoprotein, multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP) transporters have been reported to have a role to play in DRE (Margineanu and Klitgaard 2009; Sheng et al. 2018). The target hypothesis proposes the role of altered structure or function of the target of AEDs because of which the antiepileptic drugs cannot bind to its target resulting in their inability to inhibit the excessive discharge of neurons (Koubeissi 2013). The voltage gated sodium channel (VGSC) is used as the main targets for AED since they are mainly expressed in excitatory cells (Rogawski and Löscher 2004). Studies have shown that mutation in the SCN1A gene which codes for the alpha subunit of neuronal type I sodium channel has a significant role in infant myoclonic epilepsy (Møller et al. 2008). It is plausible that due to mutations in sodium channel genes, there is a loss of functional targets of the AEDs causing reduction in inhibitory sodium current and increased excitability of the whole neural network thereby contributing to epileptic discharge (Yu et al. 2006).

A more recent hypothesis is the neural network theory which suggests that changes in the brain induced upon seizure generation which include axonal sprouting, synaptic reorganization, neurogenesis, and gliosis could act as contributing factors towards abnormal network formation that consequently results in loss of endogenous inhibitory effect and also hinders the entry of AEDs into their targets, resulting in DRE (Fang et al. 2011; Yu et al. 2006). It is important to understand the underlying mechanisms of seizure generation and localization of epileptic networks involved which will help in choosing appropriate antiepileptic measures to be taken and elucidating mechanisms underlying the process of epileptogenesis (Banerjee et al. 2014). The "epileptogenic network" is defined as the area involved in generation and spread of epileptic activity (Dixit et al. 2015). Any alteration in the mechanisms that inhibit excitatory synaptic transmission or promote excitation can lead to epileptogenesis (Banerjee et al. 2013; Centeno and Carmichael 2014). The significant role of glial cells in modulating synaptic transmission and epileptogenesis cannot be underestimated, and therefore this chapter aims to discuss the fundamental aspects of glia in epilepsy pathology.

2 Reactive Gliosis in Epileptic Foci

The variety of morphological, molecular, and physiological changes that occur in glial cells, particularly astrocytes and microglia, in response to different types of neurological insults and diseases are collectively termed as gliosis (Binder and Steinhäuser 2017; Patel et al. 2019; Sofroniew 2014). Gliosis is defined by the following characteristic features: hypertrophied cell bodies and processes. Increased expression of several molecular markers for reactive glia. The markers for astrocyte activation primarily used are intermediate filament proteins glial fibrillary acidic protein (GFAP) and vimentin. Microglia activation is identified by using ionized calcium-binding adaptor molecule 1 (IBA1) and CD68. Additionally, cellular proliferation and formation of a defined scar comprising of extracellular matrix molecules like chondroitin sulfate proteoglycans (CSPGs) (Binder and Steinhäuser

2017; Sofroniew 2014; Sontheimer 2015). Upon insult the glial cells get activated and are referred to as "reactive glia" (Liddelow et al. 2017). The reactive glia may be beneficial or detrimental to the neighboring cells, and it is completely dependent on the type and severity of the injury. Reactive astrogliosis has been reported in most animal models of as well as studies based on epilepsy patient tissues (Robel et al. 2015). A study shows that gliosis induced by conditional astrocyte-specific deletion of the β1 integrin gene Itgb1 resulted in development of spontaneous, recurrent seizures in animals suggesting the role of astrogliosis in generation of epilepsy (Ortinski et al. 2010; Robel et al. 2015). Another example is seizures generated in Alexander disease which is caused by heterozygous mutations in the GFAP gene (Messing et al. 2012; Sosunov et al. 2013; Uhlmann et al. 2002). Several studies of human temporal lobe epilepsy have reported altered astrocytic properties. Hippocampal sclerosis which is also called as mesial temporal sclerosis (MTS) is widespread in patients with medically intractable temporal lobe epilepsy. MTS is characterized by neuronal cell loss in the hippocampus, occurrence of gliosis, microvascular proliferation, and synaptic reorganization (Sontheimer 2015). Gliotic scar is one of the most prominent features in chronic focal epilepsies. However, there is discrepancy in the interdependence of astrogliosis and seizures. Interestingly, using tissue resected from people with focal cortical dysplasia found an absence of gliosis (Rossini et al. 2017). Similarly, in a pilocarpine-treated mouse model of epilepsy, there was no correlation of seizure frequency with reactive gliosis. However, it was reported that a loss of GABAergic interneurons in the dentate gyrus was observed (Buckmaster et al. 2017).

Microglia, the innate immune cells of the brain also have been reported to exhibit morphological alterations after status epilepticus. Studies using human epilepsy patient samples report chronic activation and increased microglial cell number (Beach et al. 1995). A correlation is evident between the degree of microglial activation and seizure severity. Immunohistochemical expression of HLA-DR, an MHC class II cell surface receptor used to represent activated microglia, highlights the correlation of microglia activation and epilepsy frequency and duration (Boer et al. 2006). Studies involving animal models of epilepsy similarly indicated increased microglia number and reactivity following seizures (Avignone et al. 2008; Bosco et al. 2018; Matsuda et al. 2015; Tian et al. 2017). The source of microgliosis is still debatable. Some reports suggest the role of infiltrating cells from the bloodstream to CNS (Djukic et al. 2006; Flügel et al. 2001; Simard and Rivest 2004). However, few other indicate that microgliosis is due to the proliferation of resident microglia (Ajami et al. 2007). Further elaborating on this, a recent study showed that both infiltrated monocytes and activated resident microglia contributed towards the composition of microgliosis in animal model of kainic acid-induced seizures (Feng et al. 2019). Their study indicates that the two populations of cells which are microglia and infiltrating monocytes can be distinguished based on morphology and electrophysiological properties following KA-induced seizures (Feng et al. 2019).

These findings highlight that gliosis is an essential part of epilepsy histopathology and may contribute to epileptogenesis in some forms of epilepsy and not in others.

Whether gliosis is a cause or consequence of seizures is still arguable and needs to be investigated. The mechanism reactive gliosis and its role in seizure generation is an interesting and upcoming area of research (Hubbard and Binder 2016a). The role of astrocytes in maintaining ion and water homeostasis is well known, since neuronal activity greatly depends on movement of ions across the cell membrane into or out of the extracellular space (ECS), it is pertinent that any abnormality in this astrocyte function would be a cause for abnormal neuronal activity (Coulter and Steinhäuser 2015).

3 Water and K^+ Buffering

The brain tissue is very sensitive to ECS volume and osmolarity, and the ECS has a very small volume; therefore, any alterations in ion fluxes can result in dramatic changes in ion concentrations predisposing to seizure generation (Sofroniew 2014). Neurons rapidly respond to changes in the extracellular K^+ ion concentrations (Nicholson and Syková 1998). Under physiological conditions a single action potential raises extracellular K^+ concentration ($[K^+]o$) by nearly 1 mM and upon repetitive firing as in case of seizures the $[K^+]o$ raises from a resting level of ~3 to ~12 mM (Heinemann and Lux 1977). This increase requires immediate redistribution else it would result in a more positive resting potential which would affect the properties of transmembrane ion channels, receptors, and transporters. Two primary mechanisms are considered to be involved in this regulation of K^+, firstly, K^+ uptake via potassium inwardly rectifying (Kir) channels present in astrocytes and second is the spatial buffering of K^+ into the astrocyte syncytia coupled through gap junctions (GJs) (Kofuji and Newman 2004). When this buffering fails, it may contribute to epilepsy, because increased $[K^+]o$ is sufficient to induce strong epileptiform activity in hippocampal slices as suggested by studies on gliotic astrocytes from animals or humans (Gabriel et al. 2004; Moody et al. 1974; Traynelis and Dingledine 1988). In CNS, Kir4.1 is the most abundant Kir channel primarily expressed in astrocytes. Several studies using animal models and tissues from epilepsy patients have reported loss of Kir4.1 expression and function (Olsen and Sontheimer 2008; Steinhäuser et al. 2012). Downregulation of Kir4.1 has been found most significantly in the perivascular domains of astrocytic end feet in patients with mesial temporal lobe epilepsy (MTLE) and hippocampal sclerosis. Patch clamp studies which confirm a reduction in inwardly rectifying K^+ currents in astrocytes of samples obtained from drug resistant TLE-HS (Heuser et al. 2012). Deletion of astrocyte specific Kir4.1 impairs K^+ uptake and causes ataxia, seizures, and premature death in animal models (Djukic et al. 2007). KCNJ10 gene that encodes Kir4.1 has been associated with seizure susceptibility in humans and mice by quantitative trait loci studies (Buono et al. 2004). Kir4.1 is not only involved in K^+ clearance but also contributes in setting the resting membrane potential of astrocytes. The potential of astrocyte membrane is used to generate thermodynamic energy for the Na^+-dependent electrogenic glutamate transporters used to clear glutamate. Study on cultured cortical astrocytes showed that RNAi-mediated knockdown of Kir4.1 decreased uptake of

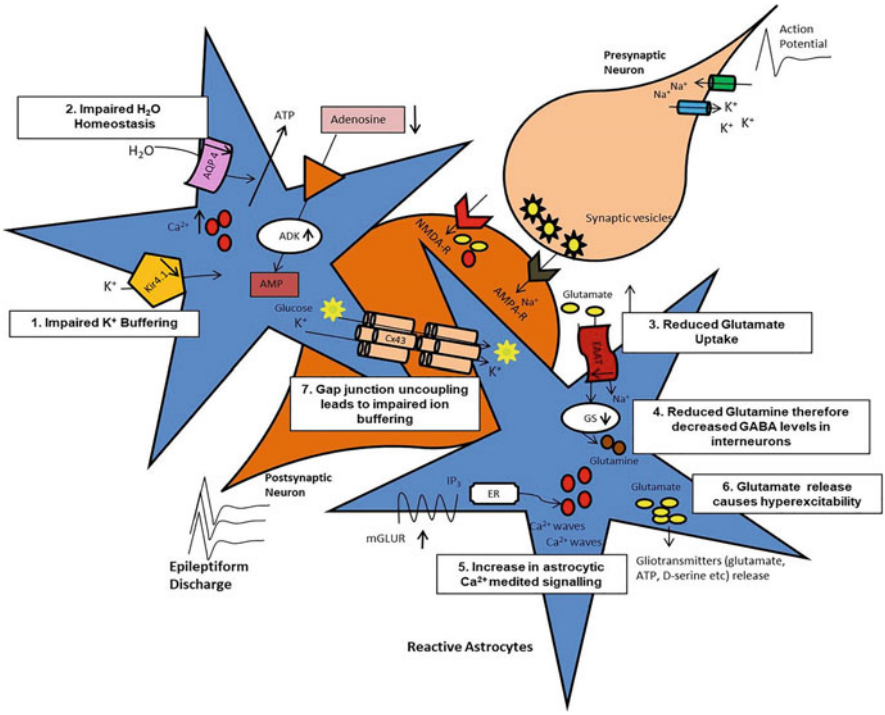

Fig. 2 Schematic representation of altered astrocyte functions in epilepsy. The diagram provides an overview of the impaired water and Ion buffering, reduced glutamate clearance, role of calcium signaling in gliotransmission, and gap junction uncoupling of all of which are instrumental in epileptogenesis

both K^+ and glutamate (Kucheryavykh et al. 2007). Studies highlight that seizure associated reactive astrocytes show reduced expression and function of Kir4.1 and consequently lead to impaired clearance of extracellular K^+ and glutamate contributing towards neuronal hyperexcitability (Fig. 2).

The movement of ions across the cell membrane along with transmembrane water flux is necessary to maintain osmotic balance (Patel et al. 2019). Consequently, water flux is crucial in determining neuronal activity since it changes the composition and size of the ECS. The aquaporins (AQPs) are a family of membrane proteins which are crucial for the transport of water across the membrane. AQPs are small hydrophobic integral membrane proteins (~30 kDa monomer) responsible for bidirectional water transport depending on osmotic gradients (Hubbard and Binder 2016d). There are several AQPs; however, AQP4 is of special interest since they are highly expressed by glial cells in the brain and spinal cord. The neuropil and astrocytic end feet are rich in AQP4 where they connect with the blood vessels (Papadopoulos and Verkman 2013). Altered AQP4 expressions have been reported in several cases of epilepsy both in animal models and patients (Binder et al. 2012). Loss of AQP4 expression in astrocyte end feet associated with vessels was reported

in patients with MTLE-HS (Eid et al. 2008b). Reports from focal cortical dysplasia and epilepsy (FCD type IIB) studies showed upregulated AQP4 in the neuropil and around dysplastic neurons, but expression around blood vessels was reduced (Medici et al. 2011). Dystrophin- associated protein complex which is composed of α-syntrophin or dystrophin anchors AQP4 to the astrocyte membrane and using a dystrophin-null transgenic mice showed a decrease in AQP4 channels (Vajda et al. 2002). A study using kainate model of MTLE showed that AQP4 mislocalization occurs prior to the chronic phase of disorder suggesting its role in epileptogenesis (Alvestad et al. 2013). There are reports which show the presence of single-nucleotide polymorphisms (SNPs) in AQP4 and KCNJ10 genes in MTLE-HS and antecedent febrile seizures (FS) patients and report that one of the SNPs had a role in resistance to seizures (Heuser et al. 2010). There is evidence which suggest that the loss or mislocalization of AQP4 channels along with decreased expression of Kir channels in MTLE are causal for impaired K^+ buffering and increased seizure susceptibility (Amiry-Moghaddam et al. 2003; Binder et al. 2006). Both, AQP4 and Kir4.1 colocalize in astrocyte end feet and the flux of ions and water through them and together regulate K^+ and water homeostasis (Nagelhus et al. 2004). Certainly, any loss of function of either Kir4.1 or AQP4 would result in enhanced seizure susceptibility (Lee et al. 2012) (Fig. 2). Along with K^+ buffering astrocytes regulate the concentration of a very important molecule, i.e., glutamate which acts as an excitatory neurotransmitter. Abnormal clearance of glutamate results in uncontrolled neuronal firing and seizure generation (Verkhratsky and Nedergaard 2018).

4 Glutamate Release and Metabolism

Increase in extracellular glutamate is a characteristic feature of animal and human epileptic tissues. Several lines of evidence suggest this causes neurotoxicity and seizures (During and Spencer 1993). Uptake of glutamate is performed by Na^+-coupled electrogenic excitatory amino acid transporters, EAAT1 and EAAT2. These transporters import one glutamate along with three Na^+ ions along with simultaneous release of one K^+ ion (Verkhratsky and Nedergaard 2018). In rodents, EAAT1 is known as glutamate aspartate transporter (GLAST) and EAAT2 as glutamate transporter 1 (GLT1), which is the primary astrocyte-specific glutamate transporter in the adult brain (Hubbard and Binder 2016e). The glutamate transporters are bidirectional; therefore, glutamate taken up by the astrocytes is rapidly converted or degraded. There are two major mechanisms by which intracellular glutamate is reduced: first is the glutamate dehydrogenase-enabled conversion to α-ketoglutarate which is an intermediary product of the tricarboxylic acid (TCA) cycle and thus provides metabolic fuel (Vandenberg and Ryan 2013). Second is the glutamate-glutamine cycle which is used to convert glutamate to glutamine catalyzed by glutamine synthetase (GS), a keystone enzyme primarily localized in astrocytes in the brain. The glutamine-glutamate cycle provides precursor for neuronal glutamate (Tian et al. 2005) and for GABA the inhibitory neurotransmitter in interneurons, where glutamate is decarboxylated by glutamate decarboxylase (GAD)

to GABA (Jiang et al. 2012; Kaczor et al. 2015). Many studies report that patients with temporal lobe epilepsy (TLE) have oddly high concentrations of extracellular glutamate which is greater in patients with HS (gliosis) (Eid et al. 2013; Petroff et al. 2002). Several studies have reported altered expressions of EAAT1 and EAAT2 in different epilepsy pathologies like MTLE-HS (Eid et al. 2008b) and FCD (Campbell and Hablitz 2008; Campbell et al. 2014); however, some studies reported no change (Kim et al. 2004). Decreased expression of the transporters is reported in patients with MTLE-HS (Sarac et al. 2009) and FCD (Ulu et al. 2010) in the CA1 region of hippocampus. In a cortical dysplasia model, inhibition of transporters resulted in opening of neuronal *N*-methyl-D-aspartate (NMDA) receptors, prolonged synaptic currents, and reduced the threshold for the initiation of epileptic activity (Campbell and Hablitz 2008) highlighting the crucial role of glutamate clearance by glial glutamate transporter (Lee et al. 2008; Tanaka et al. 1997). The b-lactam antibiotics increase glutamate uptake in astrocytes by activating EAAT2 promoter by NF-kB dependent mechanism (Rothstein et al. 2005). GS enzyme is crucial for the conversion of glutamine and studies using hippocampal tissue from TLE patients show a significant decline in the activity of GS enzyme along with a considerable loss of GS in astrocytes (Binder and Steinhäuser 2006; Eid et al. 2004; van der Hel et al. 2005). Thus, both downregulated GS expression and function along with reduced expression and function of astrocytic glutamate transporters have been reported in hippocampus of patients with TLE which together may account for the excessive glutamate levels in these patients (Eid et al. 2008a; Schousboe et al. 2014) (Fig. 2).

Metabotropic glutamate receptors (mGluRs), which are G protein-coupled receptors (GPCRs also have an important function in glutamate regulation). Astrocytes primarily express mGluR3 and mGluR5 receptors which regulate astrocytic functions as observed in epilepsy (Aronica et al. 2000, 2003; Kandratavicius et al. 2013). Various studies including patients and murine model of epilepsy have shown differential expression of mGluR5 (Aronica et al. 2000; Hanak et al. 2019). Genetic deletion of astrocytic mGluR5 has been shown to decrease glutamate clearance via glutamate transporters indicating their function in regulating glutamate transporters in epileptogenesis (Bianchi et al. 2012; Notenboom et al. 2006; Umpierre et al. 2019). Interestingly, there is evidence that activation of mGluR5 may increase excitability (Bianchi et al. 2012; Peterson and Binder 2020). In pilocarpine animal model of epilepsy, it is observed that mGluR5 activation after seizure generation increased astrocytic calcium signaling (Ding et al. 2007; Peterson and Binder 2020).

Astrocytes are not only involved in glutamate homeostasis; they are also responsible for maintaining the energy homeostasis in the brain that is crucial for optimal synaptic activity (Magistretti and Allaman 2015). The increased energy requirement by astrocytes prompts the uptake of glucose from the surrounding blood vessels through the membrane expressed glucose transporter GLUT1. Glucose undergoes glycolytic oxidation and is converted into pyruvate. However, the use of pyruvate through TCA cycle in astrocytes is limited. Therefore, astrocyte lactate dehydrogenase 5 (LDH5) converts the pyruvate produced by glycolysis into lactate. This lactate is then shuttled through monocarboxylate transporters (MCTs) to neurons,

where LDH1 drives the conversion of lactate to pyruvate which is then utilized as an energy substrate via the TCA cycle. This is often called the astrocyte neuron lactate shuttle (ANLS) pathway (Hubbard and Binder 2016e; Schousboe et al. 2014). Astrocytes are the storehouse for glycogen. Upon persistent neuronal activity, glycogen is used as an energy source; therefore, any disturbances in this metabolic pathway may tamper neuronal excitability. The use of a ketogenic diet which employs a high-fat and low-carbohydrate diet has successfully been used in controlling refractory seizures. It alters the energy metabolism probably by altering the ANLS pathway (Clasadonte and Haydon 2012; Hubbard and Binder 2016e). A study proposed that lactate from astrocytes acts as a source of ictogenic pyruvate in neurons because inhibition of LDH in astrocytes reduced neuronal excitability. Notably, an analogue of stiripentol, which is a clinically used antiepileptic drug is also an inhibitor of LDH and has been shown to potentially suppress seizures in kainic acid mice model of epilepsy (Sada et al. 2015). Astrocytes are key players in maintaining neuronal energy demands by providing energy-substrates. Furthermore, support neurotransmitter production by providing amino acid precursors (Patel et al. 2019). Thus, astrocytes are vital in sustaining normal excitation–inhibition balance. Astrocytes not only uptake glutamate but also respond to signals and release molecules called as "gliotransmitters" which include glutamate, ATP, D-serine, etc. by which they modulate synaptic transmission. The mechanism of their release greatly depends on calcium signaling (Araque et al. 2014; Parpura et al. 2012).

5 Gliotransmission: Role of Ca^{2+} Signaling

The release of several molecules from astrocytes which act as neurotransmitters and neuromodulators of neurons is called "gliotransmission" (Araque et al. 2014). The major gliotransmitters are glutamate, ATP, and adenosine. The gliotransmitters can effectively induce neuronal excitability therefore may have a significant role in epileptogenesis (Verkhratsky et al. 2014; Volterra and Meldolesi 2005). As described previously, glutamate released from astrocytes has multiple effects on neuron function which includes regulating neuronal excitability and synchronization, synaptic plasticity, and modulating GABAergic neurotransmission and production of slow inward currents in neurons (Devinsky et al. 2013; Eid et al. 2016). Solute carriers (SLCs) are transmembrane transporters which have been considered as drug targets owing to their role in movement of several molecules like nutrients, ions, metabolites, and drugs across membranes (Hu et al. 2020). Alterations in various SLCs have been reported in epilepsy patients as well as rodent models (Dikow et al. 2014; Mattison et al. 2018; Patching 2017; Stergachis et al. 2019; Zhang et al. 2019). SLC12A2 known as Na-K-Cl cotransporter (NKCC1) mediates chloride ion (Cl^-) uptake and supports depolarizing responses to GABA, while SLC12A5 also known as K-Cl co-transporter (KCC2) has a fundamental role in fast maintaining a hyperpolarizing gradient by extruding Cl^-. Alterations in the equilibrium of SLC12A2 and SLC12A5 activity are associated with epileptogenesis and therefore are extensively being studied to explore new therapeutic targets of epilepsy

(Löscher et al. 2013; Lykke et al. 2016; Moore et al. 2017; Spiciarich et al. 2019). The SLC12A2 inhibitor bumetanide has been shown to exert antiepileptic effects (Gharaylou et al. 2019). ATP is another critical gliotransmitter released by astrocytes. Astrocytes express various ion channels and G protein-coupled receptors. Purinergic signaling has pro-hyperexcitability consequences depending upon glutamate release, production of pro- inflammatory factors, alteration of the ECM, and synaptogenesis (Bowser and Khakh 2004; Zhang et al. 2003). However, adenosine unlike ATP mediates suppression of synaptic activity by activating presynaptic and postsynaptic G protein-coupled adenosine A1 receptors (A1Rs) which upon activation increase the efflux of K^+ through G protein-coupled Kir channels resulting in suppressed excitatory synaptic transmission (Boison 2016). These A1Rs have been reported to be downregulated in TLE patients as well as rodent models of epilepsy (Gomes et al. 2011). Due to this action, adenosine is also known as an endogenous anticonvulsant (During and Spencer 1992). Certain studies have indicated the role of astrocyte associated adenosine cycle in epilepsy (Boison 2016). ATP released by astrocytes is converted to adenosine in the ECS and taken up by astrocytic nucleoside transporters and subsequently phosphorylated by adenosine kinase (ADK) primarily expressed in astrocytes. Animal and human studies on epilepsy show altered ADK function indicating that adenosine deficiency may be a contribute towards for seizure generation (Aronica et al. 2011; Boison 2016) (Fig. 2).

The release of gliotransmitters is suggested to be calcium-dependent (Parpura et al. 1994). There are several lines of evidence that suggest that the release from astrocytes is similar to vesicular release of neurotransmitters from neurons (Carmignoto and Haydon 2012; Hubbard and Binder 2016c). Several studies suggest that in epilepsy the astrocyte calcium signaling is altered which may account for disturbed gliotransmitter release, including glutamate and ATP (Hubbard and Binder 2016b). The knowledge about the role of calcium mediated gliotransmitter release in epilepsy is not so well proved. However, there are reports from human tissues and animal models which suggest dysregulation of calcium channels and subsequent increase in astrocytic calcium signaling may have a role to play in hyperexcitability and seizure generation (Carmignoto and Haydon 2012; Sun et al. 2002). Several in vitro and in situ models of epilepsy have also highlighted the effect of astrocytic calcium signaling (Navarrete et al. 2013). Calcium imaging along with electrophysiological studies have shown increased calcium levels in cortical and hippocampal slices obtained from tissues resected from epileptic patients (Araque et al. 1998). Astrocytes cultured from cortical tissue of TLE patients revealed that intracellular calcium levels were elevated in an IP_3-dependent manner upon addition of albumin. Study on CA1 pyramidal neuron highlighting the calcium-dependent mechanism of release of glutamate, slow inward currents (SICs), and associated epileptiform discharges are indicative of the role of astrocytic glutamate on ionotropic glutamate receptors in neurons (Ding et al. 2007). Increased astrocytic Ca^{2+} signals promote neuronal excitotoxicity after status epilepticus as observed by two-photon microscopy after pilocarpine-induced status epilepticus (Hubbard and Binder 2016c; Orellana and Stehberg 2014). Studies have evidenced that upon adding calcium chelators, mGLUR5 antagonists, and NMDA receptor antagonists after SE the

neuronal death was reduced (Hubbard and Binder 2016e). Owing to its significance, intracellular calcium levels in astrocytes may provide novel therapeutic target for epilepsy. Additional studies are required to fully understand the role of calcium signaling in synaptic function, hyperexcitability, seizure generation, and epileptiform activity. The glial cells have a unique communication ability since they are connected with each other by gap junctions. This cell-to-cell contact allows the propagation of calcium waves, exchange of ions, and small molecules, thereby allowing homeostasis and spread of signals in the glial syncytium (Devinsky et al. 2013; Hubbard and Binder 2016c; Verkhratsky and Nedergaard 2018).

6 Cell-Cell Communication: Role of Gap Junctions

Connexins (CXs) include a highly conserved protein family encoded by 21 genes in humans and 20 in mice and having orthologs in other vertebrate species (Orellana et al. 2016). Each hemichannel or connexon is comprised of six connexins. These are arranged together to form a channel. Connexon of adjacent cells interact to form gap junctions. These channels communicate intra- and extracellular compartments and allow the release autocrine and paracrine signaling molecules, for example, ATP, glutamate, and NAD^+, to the extracellular milieu. These channels also enable the influx of small molecules of up to ~1.5 kDa like glucose and Ca^{2+} (Chever et al. 2014). With increasing knowledge about these channels their role in regulating and maintenance of homeostatic imbalance associated with brain disorders has been recognized. These hemichannels play diverse roles in glucose sensing, ischemic tolerance (Orellana et al. 2016), synaptic transmission (De Bock et al. 2011), chemoception, blood-brain barrier (BBB) permeability (Chever et al. 2014), and release of gliotransmitters (Mylvaganam et al. 2014). Studies have reported impaired astrocytic gap junction coupling in epilepsy (Bedner et al. 2015) (Fig. 2). Tracer diffusion experiments on hippocampal specimens from patients with hippocampal sclerosis evidenced complete loss of coupling in hippocampal. Studies conducted on animal models of epilepsy recapitulated these findings. Impaired K^+ clearance due to uncoupling is suggested have a role in neuronal death and occurrence of spontaneous seizure activity (Deshpande et al. 2017; Voss et al. 2009). Interestingly, decreased astrocyte coupling has been observed to precede apoptotic neuronal death and the onset of spontaneous seizures (Wu et al. 2015). Similar findings were described in mouse models of tuberous sclerosis complex and juvenile febrile seizures (Wallraff et al. 2006). However, contrary results were reported where increased coupling of hippocampal astrocytes was observed; this could be a compensatory mechanism for buffering glutamate and potassium levels in astrocytes (Deshpande et al. 2017; Vargas-Sánchez et al. 2018). Some authors suggest that increased coupling of reactive astrocytes may be implicated in the synchronization of hippocampal hyperactivity leading to neuronal loss and epileptogenesis (Wu et al. 2015). The knowledge about the role of astrocytic gap junctions in epilepsy is still limited. In vivo studies using acute hippocampal slices incubated in cytokines highlighted the role of pro-inflammatory cytokines in inducing uncoupling of hippocampal astrocytes

(Deshpande et al. 2017; Moinfar et al. 2014). Another study on mice showed decrease in coupling after 5 days of lipopolysaccharide injection. Furthermore, this uncoupling could be rescued upon administration of an anti-inflammatory and antiepileptic drug Levetiracetam (Giaume et al. 2010; Moinfar et al. 2014). These results give convincing evidence that inflammation has an important role in uncoupling of astrocytes which leads to epileptogenesis. A recent study reported the role of intracellular alkalization of astrocytes during epilepsy in uncoupling. This study highlighted that the alkaline pH shift in astrocytes causes gap junction uncoupling, resulting in altered K ion clearance which aggravates seizures generation (Onodera et al. 2021). Apart from cell-cell communication as mediated by gap junction in astrocytes, there is another significant role of astrocytes as they communicate between the blood capillaries and the neurons. The blood-brain barrier is surrounded by glial end feet which maintain the integrity of the BBB. However, the breach of BBB is detrimental and has been reported in epilepsy (Binder and Steinhäuser 2017; Hubbard and Binder 2016f; Patel et al. 2019).

7 Vasculature and the BBB in Epilepsy

The blood-brain barrier (BBB) is a dynamic system that separates the peripheral blood from the neural tissue. The BBB is comprised of cerebral microvascular endothelial cells which are linked by tight junction proteins which do not allow diffusion of water-soluble substances through the intracellular spaces and work in concert with other types of cells to build a unique microenvironment for proper functions of the neurons (van Vliet et al. 2007). Together, the neurovascular unit (NVU) is composed of endothelial cells, astrocytes, microglia, pericytes, neurons, the basement membrane, and other cellular component of the parenchyma in the brain (Seiffert et al. 2004). The astrocytic end feet interact with the endothelial cells on the abluminal membrane of the capillaries and maintain the BBB integrity (Seiffert et al. 2004). Pericytes and perivascular cells possesses elongated processes which intermittently surround the abluminal membrane of endothelial cells. Pericytes are known to have a role in regulating cerebral blood flow (Hubbard and Binder 2016g). Microglia act as the mediators of immune response in the brain, and neurons may regulate the local cerebral blood flow (Binder and Steinhäuser 2017; Patel et al. 2019). Several studies have reported that disruption of this BBB occurs in temporal lobe epilepsy (Seiffert et al. 2004: 130). It has been shown that seizures lead to increased vascular permeability and leukocyte infiltration along with reactive astrocytosis, inflammation, and hyperexcitability (Kim et al. 2016; Seiffert et al. 2004; van Vliet et al. 2007). However, the role of BBB disruption in being causal of epileptogenesis or a consequence is still an enigma. It needs a greater understanding of this system.

Glycoproteins and proteoglycans are released by glia and neurons form the matrix in ECS which exhibit a special lattice-like structure known as PNNs (Lau et al. 2013). Matrix- remodeling enzymes such as MMPs and tissue inhibitors of metalloproteinases (TIMPs) that are released by neurons and glial cells have a role in

remodeling the ECM (Dityatev 2010; Dubey et al. 2017). Therefore, molecules which disrupt PNNs result in altered GABAergic interneuron activity resulting in upregulated excitatory synaptogenesis consequently contribute towards epileptogenesis (Arranz et al. 2014; Pollock et al. 2014; van Vliet et al. 2007). Studies have shown that in pathological conditions the ECM and PNNs undergo remodeling that can possibly cause alteration in synaptic circuits as observed in animal models of epilepsy and in human epileptic tissues (Arranz et al. 2014; Pollock et al. 2014; Rankin-Gee et al. 2015; Rempe et al. 2018; Weissberg et al. 2015; Yang et al. 2007). Upon BBB disruption, the brain gets exposed to invading cells of the immune system and serum components like albumin (Weissberg et al. 2015) which is a potent trigger for neuronal hyperexcitability (Salar et al. 2014). Albumin extravasation has been reported in animal models of TLE and human samples from patients with different drug-resistant epilepsies like TLE, focal cortical dysplasia, tuberous sclerosis complex, gangliogliomas, and vascular malformations (Löscher and Friedman 2020; van Vliet et al. 2015). Albumin is reported to activate TGFβ receptors present in astrocytes resulting in reduced expression of Kir channels, AQP4, glutamate transporters, and gap junction proteins (Kim et al. 2017). Activation of TGFβ signaling pathway also results in aberrant neurogenesis, increased excitatory synaptogenesis, and pro-inflammatory molecule release from astrocytes which may account for damage to neurons and other nearby cells (Merlini et al. 2021; Patel et al. 2019; Salar et al. 2014). Albumin is considered to cause drug resistance by interacting with drugs and making them less available for binding and altering their efficiency (Ivens et al. 2007). Losartan which is a US-FDA approved drug is shown to inhibit TGFβ signaling. Reports have highlighted that administration of Losartan decreased albumin-induced chronic seizures and inhibited epileptogenesis in rat models of epilepsy(Kim et al. 2012, 2017; Patel et al. 2019) (Fig. 3). Conclusively it may be stated that the role of ECM remodeling and altered properties of the BBB significantly contribute to epileptogenesis. Inflammation is a crucial contributor of epileptogenesis. Disruption of the BBB resulting in inflammation has a great impact on the progression of epileptogenesis (Farina et al. 2007). Thereby it becomes mandatory to understand the underlying inflammatory mechanisms in epilepsy.

8 Glia-Mediated Neuroinflammation

Neuroinflammation is a physiological process elicited upon alteration of homeostasis which may arise due to any injury, infection, or dysregulated cellular functions. In the CNS, neuroinflammation is regulated by astrocyte and microglia primarily along with neurons and peripheral immune cells. This dynamic process encompasses cellular proliferation, migration, release of several factors like cytokines, chemokines, complement components, exhibition of several surface proteins like pattern recognition receptors, and regulation of different signal pathways (Streit et al. 2004; Woodcock and Morganti-Kossmann 2013). Studies including both human patient samples and animal models of epilepsy indicate that neuroinflammation may

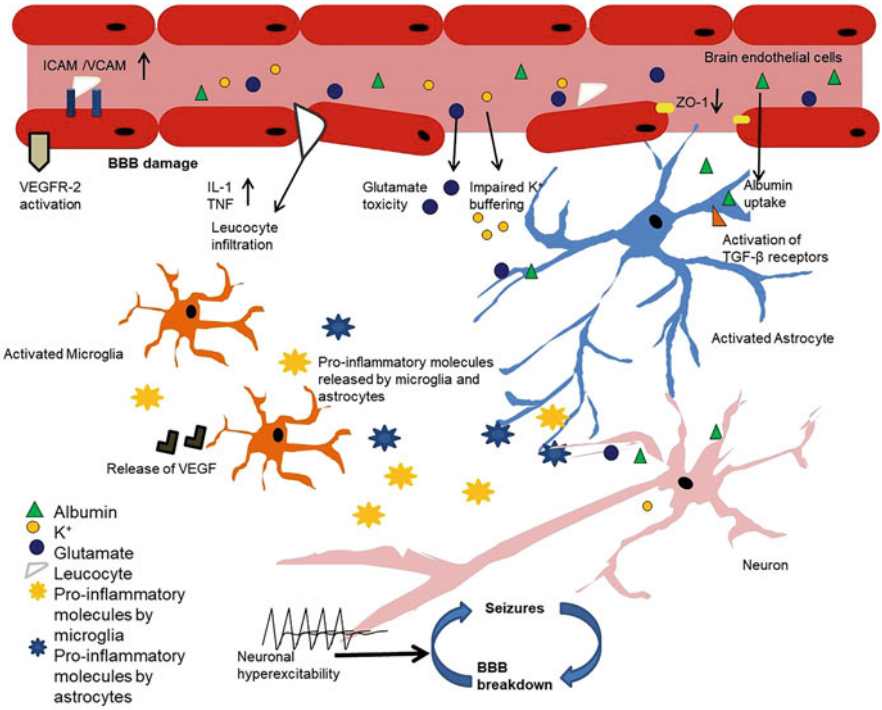

Fig. 3 Schematic representation of role of glia in BBB dysfunction and inflammation in epilepsy. The integrity of BBB is compromised resulting in seizure generation which further adds to disrupting the BBB. Inflammation is associated with epileptogenesis and BBB breakdown

contribute towards epilepsy pathologies (Choi et al. 2011; Ravizza et al. 2008; Vezzani et al. 2011b, 2013a). However, it is not very well understood whether neuroinflammation causes epileptogenesis or exacerbates the damage after seizure generation. The inflammatory response after seizure onset includes activation of glial cells, secretion of inflammatory mediators which include cytokines such as the pro-inflammatory (interleukin-1, interleukin-6, tumor necrosis factor), the anti-inflammatory cytokines (IL-4, interleukin-10, and TGF-beta), and chemokines (Aronica et al. 2011; Griffiths et al. 2009; Vezzani et al. 2013b). These inflammatory responses have been known to increase neuronal hyperactivities leading to increased neuronal cell death during epilepsy (Fig. 3) (Vezzani and Viviani 2015).

The proinflammatory mediators released from reactive astrocytes and microglia are reported to aggravate seizure activity and excitotoxicity in epilepsy (Ding et al. 2014; Miskin and Hasbani 2014). Inflammatory pathway activation is reported to reduce seizure threshold in neurons and thus has an important role in generation and recurrence of seizure (Vezzani et al. 2011a, 2013a, b). Studies indicate the role of cytokines in increasing neuronal excitability by inducing transcriptional downregulation of glutamate transporter GLT-1 in astrocytes. Cytokines also promote release of ATP, glutamate, glycine, and D-serine from astrocytes, which

enhance neuronal glutamatergic transmission (Fabene et al. 2010; Fang et al. 2012). Perivascular astrocytes and microglia may release cytokines which contribute towards BBB dysfunction in epilepsy (Vezzani et al. 2011b, 2013b). Additionally, inflammatory signaling causes the upregulation of adhesion molecules in endothelial cells which promotes recruitment of circulating leukocytes facilitating the breakdown of tight junctions leading to BBB damage (Vezzani et al. 2013a). Several studies point towards the crucial role of chemokines in controlling acute and chronic neuroinflammation in epilepsy (Xu et al. 2012). Studies conducted on experimental models and TLE patient tissues provide indicate upregulated chemokine signaling components (Kan et al. 2012; Roseti et al. 2013) Studies on KA-induced seizures model of epilepsy show release of various chemokines like CCL2, CCL3, CCL5, CCL10, and CXCL10. These findings are consistent with reports from brain tissue of epilepsy patients (Wu et al. 2008; Choi et al. 2009). A recent study highlighted the role of CCL2 in monocyte infiltration into the CNS, through CCR2 activation after seizures (Tian et al. 2017).

In epilepsy, injured or activated brain cells exhibit endogenous molecules (i.e., damage-associated molecular patterns [DAMPs] such as HMGB1, S100 proteins, adenosine triphosphate (ATP), products of extracellular matrix degradation, and IL-1b which are recognized by microglia and astrocytes thereby eliciting an immune response) (Ravizza et al. 2008, 2005). Animal studies highlight the occurrence of an inflammatory response and glial activation after *status epilepticus* in the developing hippocampus (Crespel et al. 2002) and during postnatal development (Rizzi et al. 2003). Similar findings are evidenced in tissues obtained from medial temporal lobe epilepsy with hippocampal sclerosis patients (Seifert et al. 2010). NF-kB-dependent transcriptional upregulation of various inflammatory genes has been reported in glial cells (Vezzani et al. 2011b, c; Vezzani and Friedman 2011).

Microglia selectively express the fractalkine receptor (CX3CR1) in CNS. Fractalkine (CX3CL1) is a chemotactic cytokine predominantly expressed by neurons (Cardona et al. 2006). There are reports which confirm the upregulated expression of CX3CL1 in the serum and cerebrospinal fluid of epileptic patients and in a lithium-pilocarpine rat model of epilepsy (Ali et al. 2015). CX3CL1 has been shown to control GABA-evoked currents in brain tissue obtained from epilepsy patients along with elevated CX3CR1 expression in microglial cells (Kan et al. 2012). Neurotoxicity has been associated with prolonged inflammatory responses as in case of status epilepticus in epilepsy (Bezzi et al. 2001; Henneberger and Steinhäuser 2016). The role of immune response mediators like TLR4, ATF3, and IL-8 in seizure generation and inflammation in epilepsy has also been reported in brain tissues resected from patients with epilepsy (Pernhorst et al. 2013; Walker and Sills 2012). The understanding of inflammatory mechanisms underlying generation of seizures or a consequence of seizure is crucial to provide better therapeutic approach for epilepsy pathology (Goldberg and Coulter 2013).

In a study on pediatric drug refractory epilepsy, seizure control was achieved upon anti-inflammatory treatment, thus providing evidence towards the role of inflammatory mediators in seizure generation (Aronica et al. 2012; Vezzani et al. 2000). Additionally, there are reports that show that application of microglial

inhibitors, such as minocycline and macrophage inhibitory factor (MIF) reduced neurodegeneration in epilepsy (Rogove and Tsirka 1998; Wang et al. 2012).

9 Future Perspectives

The contribution of glial cells to epileptogenesis has been unequivocally demonstrated in several studies using resected tissues from epilepsy patients as well as several animal models of epilepsy (Clarke and Barres 2013). However, there are several crucial questions which need to be addressed. Firstly, the different functions of astrocytes and microglia in regulating synaptic transmission, ion homeostasis, and glutamate clearance may be subjective to the pathophysiology of other forms of epilepsy. Therefore, it is important to look for changes in glial functions in different forms of epilepsy pathologies which have not been well explored yet unlike MTLE and focal cortical dysplasia. Secondly, in lieu of the crucial role of astrocytes in modulating the BBB microenvironment, it becomes of great importance to focus epilepsy research on this. Since, the gliovasculature junction is not so well understood; it provides an open ground for future research to unravel the underlying mechanisms of epileptogenesis. The role of BBB in epileptogenesis is very intriguing, and the cellular and molecular roles of the gliovascular junction in regulating metabolic homeostasis and epileptogenesis are not explored as much; therefore, it is an important area for future studies to be carried out (Löscher and Friedman 2020). Third, an important recent development is the recognition of structural and functional heterogeneity of astrocytes and microglia. The discovery of NG2 glia is more recent and requires future studies for a better understanding. The understanding of microglia polarization states, and the recently described disease-associated microglia (DAM) phenotypes require more characterization in epilepsy (Keren-Shaul et al. 2017). Fourth, another interesting area of research can be the glial interaction between different cell types like microglia, astrocytes, oligodendrocytes, endothelial cells, and pericytes in different regions of the brain. This will provide a broader insight into the glial communication. Fifth, despite a great deal of knowledge and research about epilepsy, there is still a growing demand for development of new and better AEDs which may have lesser side effects, improved antiepileptogenic effects, and increased tolerability. There are various potential targets like IL-1β, TGF-β, mTOR signaling pathway, and many others which have been described to have important role epileptogenesis and may be harnessed for developing better AEDs (Kambli et al. 2017). Another important research area is the employment of genetic tools in understanding the genetic variants associated with different form of epilepsies. To improve diagnosis and treatment, it is essential to incorporate genetic testing and identifying genetic biomarkers in different subtypes of DRE pathologies (Balestrini and Sisodiya 2018; Thakran et al. 2020).

Therefore, future research should be based on using different approaches and strategies to explore new therapeutic targets for controlling seizures and epileptogenesis. Further studies on the role of glia in epilepsy should enable the

Fig. 4 Summarising the role of glial cells in epileptogenesis and possible therapeutic targets (Aronica et al. 2001; Dixit et al. 2016; Kambli et al. 2017)

identification of novel molecular targets which might unravel new possibilities for the development of alternative antiepileptic therapies (Fig. 4).

Acknowledgement This work was supported by the Magnetoencephalography (MEG) Resource Facility grant funded by Department of Biotechnology, Ministry of Science & Technology, Govt. of India [Grant: BT/MED/122/SP24580/2018].

References

Nagelhus EA, Mathiisen TM, Ottersen OP (2004) Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. Neuroscience 129:905–913

Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM (2007) Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci 10:1538–1543

Ali I, Chugh D, Ekdahl CT (2015) Role of fractalkine-CX3CR1 pathway in seizure-induced microglial activation, neurodegeneration, and neuroblast production in the adult rat brain. Neurobiol Dis 74:194–203. https://doi.org/10.1016/j.nbd.2014.11.009. Epub 2014 Nov 21. PMID: 25461978

Alvestad S, Hammer J, Hoddevik EH, Skare Ø, Sonnewald U, Amiry-Moghaddam M, Ottersen OP (2013) Mislocalization of AQP4 precedes chronic seizures in the kainate model of temporal lobe epilepsy. Epilepsy Res 105:30–41

Amiry-Moghaddam M, Williamson A, Palomba M, Eid T, de Lanerolle NC, Nagelhus EA, Adams ME, Froehner SC, Agre P, Ottersen OP (2003) Delayed K^+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of alpha-syntrophin-null mice. Proc Natl Acad Sci U S A 100:13615–13620

Araque A, Carmignoto G, Haydon PG, Oliet SH, Robitaille R, Volterra A (2014) Gliotransmitters travel in time and space. Neuron 81:728–739

Araque A, Sanzgiri RP, Parpura V, Haydon PG (1998) Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. J Neurosci 18:6822–6829

Aronica E, Gorter JA, Ijlst-Keizers H, Rozemuller AJ, Yankaya B, Leenstra S, Troost D (2003) Expression and functional role of mGluR3 and mGluR5 in human astrocytes and glioma cells: opposite regulation of glutamate transporter proteins. Eur J Neurosci 17:2106–2118

Aronica E, Ravizza T, Zurolo E, Vezzani A (2012) Astrocyte immune responses in epilepsy. Glia 60:1258–1268

Aronica E, van Vliet EA, Mayboroda OA, Troost D, da Silva FH, Gorter JA (2000) Upregulation of metabotropic glutamate receptor subtype mGluR3 and mGluR5 in reactive astrocytes in a rat model of mesial temporal lobe epilepsy. Eur J Neurosci 12:2333–2344

Aronica E, Yankaya B, Jansen GH, Leenstra S, van Veelen CW, Gorter JA, Troost D (2001) Ionotropic and metabotropic glutamate receptor protein expression in glioneuronal tumours from patients with intractable epilepsy. Neuropathol Appl Neurobiol 27:223–237

Aronica E, Zurolo E, Iyer A, de Groot M, Anink J, Carbonell C, van Vliet EA, Baayen JC, Boison D, Gorter JA (2011) Upregulation of adenosine kinase in astrocytes in experimental and human temporal lobe epilepsy. Epilepsia 52:1645–1655

Arranz AM, Perkins KL, Irie F, Lewis DP, Hrabe J, Xiao F, Itano N, Kimata K, Hrabetova S, Yamaguchi Y (2014) Hyaluronan deficiency due to Has3 knock-out causes altered neuronal activity and seizures via reduction in brain extracellular space. J Neurosci 34:6164–6176

Avignone E, Ulmann L, Levavasseur F, Rassendren F, Audinat E (2008) Status epilepticus induces a particular microglial activation state characterized by enhanced purinergic signaling. J Neurosci 28:9133

Balestrini S, Sisodiya SM (2018) Pharmacogenomics in epilepsy. Neurosci Lett 667:27–39

Banerjee J, Chandra SP, Kurwale N, Tripathi M (2014) Epileptogenic networks and drug-resistant epilepsy: present and future perspectives of epilepsy research-utility for the epileptologist and the epilepsy surgeon. Ann Indian Acad Neurol 17:S134–S140

Banerjee J, Tripathi M, Sarat Chandra P (2013) Understanding complexities of synaptic transmission in medically intractable seizures: a paradigm of epilepsy research. Indian J Neurosurg 02:071–076

Beach TG, Woodhurst WB, MacDonald DB, Jones MW (1995) Reactive microglia in hippocampal sclerosis associated with human temporal lobe epilepsy. Neurosci Lett 191:27–30

Bedner P, Dupper A, Hüttmann K, Müller J, Herde MK, Dublin P, Deshpande T, Schramm J, Häussler U, Haas CA, Henneberger C, Theis M, Steinhäuser C (2015) Astrocyte uncoupling as a cause of human temporal lobe epilepsy. Brain 138:1208–1222

Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta G, Kollias G, Meldolesi J, Volterra A (2001) CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. Nat Neurosci 4:702–710

Bianchi R, Wong RKS, Merlin LR (2012) Glutamate receptors in epilepsy: group I mGluR-mediated epileptogenesis. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW,

Delgado-Escueta AV (eds) Jasper's basic mechanisms of the epilepsies. National Center for Biotechnology Information (US), Bethesda, MD
Binder DK, Nagelhus EA, Ottersen OP (2012) Aquaporin-4 and epilepsy. Glia 60:1203–1214
Binder DK, Steinhäuser C (2006) Functional changes in astroglial cells in epilepsy. Glia 54:358–368
Binder DK, Steinhäuser C (2017) Role of astrocyte dysfunction in epilepsy☆. In: Reference module in neuroscience and biobehavioral psychology. Elsevier, Amsterdam
Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT (2006) Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. Glia 53:631–636
Boer K, Spliet WG, van Rijen PC, Redeker S, Troost D, Aronica E (2006) Evidence of activated microglia in focal cortical dysplasia. J Neuroimmunol 173:188–195
Boison D (2016) Adenosinergic signaling in epilepsy. Neuropharmacology 104:131–139
Bosco DB, Zheng J, Xu Z, Peng J, Eyo UB, Tang K, Yan C, Huang J, Feng L, Wu G, Richardson JR, Wang H, Wu LJ (2018) RNAseq analysis of hippocampal microglia after kainic acid-induced seizures. Mol Brain 11:34
Bowser DN, Khakh BS (2004) ATP excites interneurons and astrocytes to increase synaptic inhibition in neuronal networks. J Neurosci 24:8606–8620
Buckmaster PS, Abrams E, Wen X (2017) Seizure frequency correlates with loss of dentate gyrus GABAergic neurons in a mouse model of temporal lobe epilepsy. J Comp Neurol 525:2592–2610
Buono RJ, Lohoff FW, Sander T, Sperling MR, O'Connor MJ, Dlugos DJ, Ryan SG, Golden GT, Zhao H, Scattergood TM, Berrettini WH, Ferraro TN (2004) Association between variation in the human KCNJ10 potassium ion channel gene and seizure susceptibility. Epilepsy Res 58:175–183
Campbell SL, Hablitz JJ (2008) Decreased glutamate transport enhances excitability in a rat model of cortical dysplasia. Neurobiol Dis 32:254–261
Campbell SL, Hablitz JJ, Olsen ML (2014) Functional changes in glutamate transporters and astrocyte biophysical properties in a rodent model of focal cortical dysplasia. Front Cell Neurosci 8:425
Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9(7):917–924. https://doi.org/10.1038/nn1715. Epub 2006 Jun 18. PMID: 16732273
Carmignoto G, Haydon PG (2012) Astrocyte calcium signaling and epilepsy. Glia 60:1227–1233
Centeno M, Carmichael DW (2014) Network connectivity in epilepsy: resting state fMRI and EEG-fMRI contributions. Front Neurol 5:93
Chever O, Lee C-Y, Rouach N (2014) Astroglial connexin43 hemichannels tune basal excitatory synaptic transmission. J Neurosci 34:11228
Choi J, Nordli DR Jr, Alden TD, DiPatri A Jr, Laux L, Kelley K, Rosenow J, Schuele SU, Rajaram V, Koh S (2009) Cellular injury and neuroinflammation in children with chronic intractable epilepsy. J Neuroinflammation 19(6):38. https://doi.org/10.1186/1742-2094-6-38. PMID: 20021679; PMCID: PMC2811703
Choi J, Min HJ, Shin JS (2011) Increased levels of HMGB1 and pro-inflammatory cytokines in children with febrile seizures. J Neuroinflammation 8:135
Clarke LE, Barres BA (2013) Emerging roles of astrocytes in neural circuit development. Nat Rev Neurosci 14:311–321
Clasadonte J, Haydon PG (2012) Astrocytes and epilepsy. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV (eds) Jasper's basic mechanisms of the epilepsies. National Center for Biotechnology Information (US), Bethesda, MD
Coulter DA, Steinhäuser C (2015) Role of astrocytes in epilepsy. Cold Spring Harb Perspect Med 5:a022434

Crespel A, Coubes P, Rousset MC, Brana C, Rougier A, Rondouin G, Bockaert J, Baldy-Moulinier-M, Lerner-Natoli M (2002) Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. Brain Res 952:159–169

De Bock M, Culot M, Wang N, Bol M, Decrock E, De Vuyst E, da Costa A, Dauwe I, Vinken M, Simon AM, Rogiers V, De Ley G, Evans WH, Bultynck G, Dupont G, Cecchelli R, Leybaert L (2011) Connexin channels provide a target to manipulate brain endothelial calcium dynamics and blood-brain barrier permeability. J Cereb Blood Flow Metab 31:1942–1957

Deshpande T, Li T, Herde MK, Becker A, Vatter H, Schwarz MK, Henneberger C, Steinhäuser C, Bedner P (2017) Subcellular reorganization and altered phosphorylation of the astrocytic gap junction protein connexin43 in human and experimental temporal lobe epilepsy. Glia 65:1809–1820

Devinsky O, Vezzani A, Najjar S, De Lanerolle NC, Rogawski MA (2013) Glia and epilepsy: excitability and inflammation. Trends Neurosci 36:174–184

Dikow N, Maas B, Karch S, Granzow M, Janssen JW, Jauch A, Hinderhofer K, Sutter C, Schubert-Bast S, Anderlid BM, Dallapiccola B, Van der Aa N, Moog U (2014) 3p253 microdeletion of GABA transporters SLC6A1 and SLC6A11 results in intellectual disability, epilepsy and stereotypic behavior. Am J Med Genet A 164a:3061–3068

Ding S, Fellin T, Zhu Y, Lee SY, Auberson YP, Meaney DF, Coulter DA, Carmignoto G, Haydon PG (2007) Enhanced astrocytic Ca^{2+} signals contribute to neuronal excitotoxicity after status epilepticus. J Neurosci 27:10674–10684

Ding Z, Mathur V, Ho PP, James ML, Lucin KM, Hoehne A, Alabsi H, Gambhir SS, Steinman L, Luo J, Wyss-Coray T (2014) Antiviral drug ganciclovir is a potent inhibitor of microglial proliferation and neuroinflammation. J Exp Med 211(2):189–198. https://doi.org/10.1084/jem.20120696

Dityatev A (2010) Remodeling of extracellular matrix and epileptogenesis. Epilepsia 51(Suppl 3): 61–65

Dixit AB, Banerjee J, Tripathi M, Chandra PS (2015) Presurgical epileptogenic network analysis: a way to enhance epilepsy surgery outcome. Neurol India 63:743–750

Dixit AB, Tripathi M, Chandra PS, Banerjee J (2016) Molecular biomarkers in drug-resistant epilepsy: facts & possibilities. Int J Surg (London, England) 36:483–491

Djukic B, Casper KB, Philpot BD, Chin LS, McCarthy KD (2007) Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J Neurosci 27:11354–11365

Djukic M, Mildner A, Schmidt H, Czesnik D, Brück W, Priller J, Nau R, Prinz M (2006) Circulating monocytes engraft in the brain, differentiate into microglia and contribute to the pathology following meningitis in mice. Brain 129:2394–2403

Dubey D, McRae PA, Rankin-Gee EK, Baranov E, Wandrey L, Rogers S, Porter BE (2017) Increased metalloproteinase activity in the hippocampus following status epilepticus. Epilepsy Res 132:50–58

During MJ, Spencer DD (1992) Adenosine: a potential mediator of seizure arrest and postictal refractoriness. Ann Neurol 32:618–624

During MJ, Spencer DD (1993) Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. Lancet (London, England) 341:1607–1610

Eid T, Ghosh A, Wang Y, Beckström H, Zaveri HP, Lee TS, Lai JC, Malthankar-Phatak GH, de Lanerolle NC (2008a) Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. Brain 131:2061–2070

Eid T, Gruenbaum SE, Dhaher R, Lee TW, Zhou Y, Danbolt NC (2016) The glutamate-glutamine cycle in epilepsy. Adv Neurobiol 13:351–400

Eid T, Lee TS, Wang Y, Perez E, Drummond J, Lauritzen F, Bergersen LH, Meador-Woodruff JH, Spencer DD, de Lanerolle NC, McCullumsmith RE (2013) Gene expression of glutamate metabolizing enzymes in the hippocampal formation in human temporal lobe epilepsy. Epilepsia 54:228–238

Eid T, Thomas MJ, Spencer DD, Rundén-Pran E, Lai JC, Malthankar GV, Kim JH, Danbolt NC, Ottersen OP, de Lanerolle NC (2004) Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. Lancet (London, England) 363:28–37

Eid T, Williamson A, Lee TS, Petroff OA, de Lanerolle NC (2008b) Glutamate and astrocytes—key players in human mesial temporal lobe epilepsy? Epilepsia 49(Suppl 2):42–52

Fabene PF, Bramanti P, Constantin G (2010) The emerging role for chemokines in epilepsy. J Neuroimmunol 224:22–27

Fang J, Han D, Hong J, Tan Q, Tian Y (2012) The chemokine, macrophage inflammatory protein-2γ, reduces the expression of glutamate transporter-1 on astrocytes and increases neuronal sensitivity to glutamate excitotoxicity. J Neuroinflammation 9:267

Fang M, Xi ZQ, Wu Y, Wang XF (2011) A new hypothesis of drug refractory epilepsy: neural network hypothesis. Med Hypotheses 76:871–876

Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. Trends Immunol 28:138–145

Feng L, Murugan M, Bosco DB, Liu Y, Peng J, Worrell GA, Wang HL, Ta LE, Richardson JR, Shen Y, Wu LJ (2019) Microglial proliferation and monocyte infiltration contribute to microgliosis following status epilepticus. Glia 67:1434–1448

Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J Jr, Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshe SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S (2014) ILAE official report: a practical clinical definition of epilepsy. Epilepsia 55:475–482

Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, Lagae L, Moshé SL, Peltola J, Roulet Perez E, Scheffer IE, Zuberi SM (2017) Operational classification of seizure types by the International League Against Epilepsy: position paper of the ILAE Commission for Classification and Terminology. Epilepsia 58:522–530

Flügel A, Bradl M, Kreutzberg GW, Graeber MB (2001) Transformation of donor-derived bone marrow precursors into host microglia during autoimmune CNS inflammation and during the retrograde response to axotomy. J Neurosci Res 66:74–82

Gabriel S, Njunting M, Pomper JK, Merschhemke M, Sanabria ER, Eilers A, Kivi A, Zeller M, Meencke HJ, Cavalheiro EA, Heinemann U, Lehmann TN (2004) Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. J Neurosci 24:10416–10430

Gharaylou Z, Tafakhori A, Agah E, Aghamollaii V, Kebriaeezadeh A, Hadjighassem M (2019) A preliminary study evaluating the safety and efficacy of bumetanide, an NKCC1 inhibitor, in patients with drug-resistant epilepsy. CNS Drugs 33:283–291

Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N (2010) Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 11:87–99

Goldberg EM, Coulter DA (2013) Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. Nat Rev Neurosci 14:337–349

Gomes CV, Kaster MP, Tomé AR, Agostinho PM, Cunha RA (2011) Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. Biochim Biophys Acta 1808:1380–1399

Griffiths MR, Gasque P, Neal JW (2009) The multiple roles of the innate immune system in the regulation of apoptosis and inflammation in the brain. J Neuropathol Exp Neurol 68:217–226

Hanak TJ, Libbey JE, Doty DJ, Sim JT, DePaula-Silva AB, Fujinami RS (2019) Positive modulation of mGluR5 attenuates seizures and reduces TNF-α(+) macrophages and microglia in the brain in a murine model of virus-induced temporal lobe epilepsy. Exp Neurol 311:194–204

Heinemann U, Lux HD (1977) Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. Brain Res 120:231–249

Henneberger C, Steinhäuser C (2016) Astrocytic TLR4 at the crossroads of inflammation and seizure susceptibility. J Cell Biol 215:607–609

Heuser K, Eid T, Lauritzen F, Thoren AE, Vindedal GF, Taubøll E, Gjerstad L, Spencer DD, Ottersen OP, Nagelhus EA, de Lanerolle NC (2012) Loss of perivascular Kir4.1 potassium channels in the sclerotic hippocampus of patients with mesial temporal lobe epilepsy. J Neuropathol Exp Neurol 71:814–825

Heuser K, Nagelhus EA, Taubøll E, Indahl U, Berg PR, Lien S, Nakken S, Gjerstad L, Ottersen OP (2010) Variants of the genes encoding AQP4 and Kir4.1 are associated with subgroups of patients with temporal lobe epilepsy. Epilepsy Res 88:55–64

Hu C, Tao L, Cao X, Chen L (2020) The solute carrier transporters and the brain: physiological and pharmacological implications. Asian J Pharm Sci 15:131–144

Hubbard JA, Binder DK (2016a) Chapter 2—Astrocytes in the mammalian brain. In: Hubbard JA, Binder DK (eds) Astrocytes and epilepsy. Academic, San Diego, pp 39–51

Hubbard JA, Binder DK (2016b) Chapter 3—Gliotransmitters. In: Hubbard JA, Binder DK (eds) Astrocytes and epilepsy. Academic, San Diego, pp 53–73

Hubbard JA, Binder DK (2016c) Chapter 6—Astrocyte calcium signaling. In: Hubbard JA, Binder DK (eds) Astrocytes and epilepsy. Academic, San Diego, pp 125–145

Hubbard JA, Binder DK (2016d) Chapter 8—Water channels. In: Hubbard JA, Binder DK (eds) Astrocytes and epilepsy. Academic, San Diego, pp 171–195

Hubbard JA, Binder DK (2016e) Chapter 9—Glutamate metabolism. In: Hubbard JA, Binder DK (eds) Astrocytes and epilepsy. Academic, San Diego, pp 197–224

Hubbard JA, Binder DK (2016f) Chapter 11—Gap junctions. In: Hubbard JA, Binder DK (eds) Astrocytes and epilepsy. Academic, San Diego, pp 265–289

Hubbard JA, Binder DK (2016g) Chapter 12—Blood–brain barrier disruption. In: Hubbard JA, Binder DK (eds) Astrocytes and epilepsy. Academic, San Diego, pp 291–311

Ivens S, Kaufer D, Flores LP, Bechmann I, Zumsteg D, Tomkins O, Seiffert E, Heinemann U, Friedman A (2007) TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. Brain 130:535–547

Jiang E, Yan X, Weng HR (2012) Glial glutamate transporter and glutamine synthetase regulate GABAergic synaptic strength in the spinal dorsal horn. J Neurochem 121:526–536

Kaczor P, Rakus D, Mozrzymas JW (2015) Neuron-astrocyte interaction enhance GABAergic synaptic transmission in a manner dependent on key metabolic enzymes. Front Cell Neurosci 9: 120

Kambli L, Bhatt LK, Oza M, Prabhavalkar K (2017) Novel therapeutic targets for epilepsy intervention. Seizure 51:27–34

Kan AA, van der Hel WS, Kolk SM, Bos IW, Verlinde SA, van Nieuwenhuizen O, de Graan PN (2012) Prolonged increase in rat hippocampal chemokine signalling after status epilepticus. J Neuroimmunol 245:15–22

Kandratavicius L, Rosa-Neto P, Monteiro MR, Guiot MC, Assirati JA Jr, Carlotti CG Jr, Kobayashi E, Leite JP (2013) Distinct increased metabotropic glutamate receptor type 5 (mGluR5) in temporal lobe epilepsy with and without hippocampal sclerosis. Hippocampus 23:1212–1230

Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, Itzkovitz S, Colonna M, Schwartz M, Amit I (2017) A unique microglia type associated with restricting development of Alzheimer's disease. Cell 169:1276–1290.e17

Kim JH, Je S, Petroff OA, Spencer SS, Hwang JY, Spencer DDJE (2004) Hippocampal glial density in temporal lobe epilepsy. Epilepsia 45:S33–S34

Kim SY, Buckwalter M, Soreq H, Vezzani A, Kaufer D (2012) Blood-brain barrier dysfunction-induced inflammatory signaling in brain pathology and epileptogenesis. Epilepsia 53(Suppl 6): 37–44

Kim SY, Porter BE, Friedman A, Kaufer D (2016) A potential role for glia-derived extracellular matrix remodeling in postinjury epilepsy. J Neurosci Res 94:794–803

Kim SY, Senatorov VV Jr, Morrissey CS, Lippmann K, Vazquez O, Milikovsky DZ, Gu F, Parada I, Prince DA, Becker AJ, Heinemann U, Friedman A, Kaufer D (2017) TGFβ signaling

is associated with changes in inflammatory gene expression and perineuronal net degradation around inhibitory neurons following various neurological insults. Sci Rep 7:7711
Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. Neuroscience 129:1045–1056
Koubeissi M (2013) Neuropathology of the blood-brain barrier in epilepsy: support to the transport hypothesis of pharmacoresistance. Epilepsy Curr 13:169–171
Kucheryavykh YV, Kucheryavykh LY, Nichols CG, Maldonado HM, Baksi K, Reichenbach A, Skatchkov SN, Eaton MJ (2007) Downregulation of Kir4.1 inward rectifying potassium channel subunits by RNAi impairs potassium transfer and glutamate uptake by cultured cortical astrocytes. Glia 55:274–281
Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, Moshé SL, Perucca E, Wiebe S, French J (2010) Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia 51: 1069–1077
Lau LW, Cua R, Keough MB, Haylock-Jacobs S, Yong VW (2013) Pathophysiology of the brain extracellular matrix: a new target for remyelination. Nat Rev Neurosci 14:722–729
Lee DJ, Hsu MS, Seldin MM, Arellano JL, Binder DK (2012) Decreased expression of the glial water channel aquaporin-4 in the intrahippocampal kainic acid model of epileptogenesis. Exp Neurol 235:246–255
Lee SG, Su ZZ, Emdad L, Gupta P, Sarkar D, Borjabad A, Volsky DJ, Fisher PB (2008) Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. J Biol Chem 283:13116–13123
Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B et al (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481–487
Löscher W, Friedman A (2020) Structural, molecular, and functional alterations of the blood-brain barrier during epileptogenesis and epilepsy: a cause, consequence, or both? Int J Mol Sci 21(2): 591
Löscher W, Luna-Tortós C, Römermann K, Fedrowitz M (2011) Do ATP-binding cassette transporters cause pharmacoresistance in epilepsy? Problems and approaches in determining which antiepileptic drugs are affected. Curr Pharm Des 17:2808–2828
Löscher W, Potschka H (2005) Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci 6:591–602
Löscher W, Puskarjov M, Kaila K (2013) Cation-chloride cotransporters NKCC1 and KCC2 as potential targets for novel antiepileptic and antiepileptogenic treatments. Neuropharmacology 69:62–74
Lykke K, Töllner K, Feit PW, Erker T, MacAulay N, Löscher W (2016) The search for NKCC1-selective drugs for the treatment of epilepsy: structure-function relationship of bumetanide and various bumetanide derivatives in inhibiting the human cation-chloride cotransporter NKCC1A. Epilepsy Behav 59:42–49
Magistretti PJ, Allaman I (2015) A cellular perspective on brain energy metabolism and functional imaging. Neuron 86:883–901
Margineanu DG, Klitgaard H (2009) Mechanisms of drug resistance in epilepsy: relevance for antiepileptic drug discovery. Expert Opin Drug Discov 4:23–32
Matsuda T, Murao N, Katano Y, Juliandi B, Kohyama J, Akira S, Kawai T, Nakashima K (2015) TLR9 signalling in microglia attenuates seizure-induced aberrant neurogenesis in the adult hippocampus. Nat Commun 6:6514–6514
Mattison KA, Butler KM, Inglis GAS, Dayan O, Boussidan H, Bhambhani V, Philbrook B, da Silva C, Alexander JJ, Kanner BI, Escayg A (2018) SLC6A1 variants identified in epilepsy patients reduce γ-aminobutyric acid transport. Epilepsia 59:e135–e141
Medici V, Frassoni C, Tassi L, Spreafico R, Garbelli R (2011) Aquaporin 4 expression in control and epileptic human cerebral cortex. Brain Res 1367:330–339

Merlini M, Rafalski VA, Ma K, Kim K-Y, Bushong EA, Rios Coronado PE, Yan Z, Mendiola AS, Sozmen EG, Ryu JK, Haberl MG, Madany M, Sampson DN, Petersen MA, Bardehle S, Tognatta R, Dean T, Acevedo RM, Cabriga B, Thomas R et al (2021) Microglial Gi-dependent dynamics regulate brain network hyperexcitability. Nat Neurosci 24:19–23

Messing A, Brenner M, Feany MB, Nedergaard M, Goldman JE (2012) Alexander disease. J Neurosci 32:5017–5023

Miskin C, Hasbani DM, Epilepticus S (2014) Immunologic and inflammatory mechanisms. Semin Pediatr Neurol 21(3):221–225. ISSN 1071-9091. https://doi.org/10.1016/j.spen.2014.09.001

Moinfar Z, Dambach H, Faustmann PM (2014) Influence of drugs on gap junctions in glioma cell lines and primary astrocytes in vitro. Front Physiol 5:186

Møller RS, Schneider LM, Hansen CP, Bugge M, Ullmann R, Tommerup N, Tümer Z (2008) Balanced translocation in a patient with severe myoclonic epilepsy of infancy disrupts the sodium channel gene SCN1A. Epilepsia 49:1091–1094

Moody WJ, Futamachi KJ, Prince DA (1974) Extracellular potassium activity during epileptogenesis. Exp Neurol 42:248–263

Moore YE, Kelley MR, Brandon NJ, Deeb TZ, Moss SJ (2017) Seizing control of KCC2: a new therapeutic target for epilepsy. Trends Neurosci 40:555–571

Mylvaganam S, Ramani M, Krawczyk M, Carlen PL (2014) Roles of gap junctions, connexins, and pannexins in epilepsy. Front Physiol 5:172

Navarrete M, Perea G, Maglio L, Pastor J, García de Sola R, Araque A (2013) Astrocyte calcium signal and gliotransmission in human brain tissue. Cereb Cortex (New York, NY: 1991) 23: 1240–1246

Nicholson C, Syková E (1998) Extracellular space structure revealed by diffusion analysis. Trends Neurosci 21:207–215

Notenboom RG, Hampson DR, Jansen GH, van Rijen PC, van Veelen CW, van Nieuwenhuizen O, de Graan PN (2006) Up-regulation of hippocampal metabotropic glutamate receptor 5 in temporal lobe epilepsy patients. Brain 129:96–107

Olsen ML, Sontheimer H (2008) Functional implications for Kir4.1 channels in glial biology: from K^+ buffering to cell differentiation. J Neurochem 107:589–601

Onodera M, Meyer J, Furukawa K, Hiraoka Y, Aida T, Tanaka K, Tanaka KF, Rose CR, Matsui K (2021) Exacerbation of epilepsy by astrocyte alkalization and gap junction uncoupling. J Neurosci 41:2106–2118

Orellana JA, Retamal MA, Moraga-Amaro R, Stehberg J (2016) Role of astroglial hemichannels and pannexons in memory and neurodegenerative diseases. Front Integr Neurosci 10:26

Orellana JA, Stehberg J (2014) Hemichannels: new roles in astroglial function. Front Physiol 5:193

Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon PG, Coulter DA (2010) Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. Nat Neurosci 13:584–591

Pack AM (2019) Epilepsy overview and revised classification of seizures and epilepsies. Continuum (Minneapolis, Minn) 25:306–321

Papadopoulos MC, Verkman AS (2013) Aquaporin water channels in the nervous system. Nat Rev Neurosci 14:265–277

Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG (1994) Glutamate-mediated astrocyte-neuron signalling. Nature 369:744–747

Parpura V, Heneka MT, Montana V, Oliet SH, Schousboe A, Haydon PG, Stout RF Jr, Spray DC, Reichenbach A, Pannicke T, Pekny M, Pekna M, Zorec R, Verkhratsky A (2012) Glial cells in (patho)physiology. J Neurochem 121:4–27

Patching SG (2017) Glucose transporters at the blood-brain barrier: function, regulation and gateways for drug delivery. Mol Neurobiol 54:1046–1077

Patel DC, Tewari BP, Chaunsali L, Sontheimer H (2019) Neuron-glia interactions in the pathophysiology of epilepsy. Nat Rev Neurosci 20:282–297

Pernhorst K, Herms S, Hoffmann P, Cichon S, Schulz H, Sander T, Schoch S, Becker AJ, Grote A (2013) TLR4, ATF-3 and IL8 inflammation mediator expression correlates with seizure frequency in human epileptic brain tissue. Seizure 22:675–678

Peterson AR, Binder DK (2020) Astrocyte glutamate uptake and signaling as novel targets for antiepileptogenic therapy. Front Neurol 11:1006

Petroff OA, Errante LD, Rothman DL, Kim JH, Spencer DD (2002) Glutamate-glutamine cycling in the epileptic human hippocampus. Epilepsia 43:703–710

Pollock E, Everest M, Brown A, Poulter MO (2014) Metalloproteinase inhibition prevents inhibitory synapse reorganization and seizure genesis. Neurobiol Dis 70:21–31

Ramos-Lizana J, Aguilera-López P, Aguirre-Rodríguez J, Cassinello-García E (2009) Response to sequential treatment schedules in childhood epilepsy: risk for development of refractory epilepsy. Seizure 18:620–624

Rankin-Gee EK, McRae PA, Baranov E, Rogers S, Wandrey L, Porter BE (2015) Perineuronal net degradation in epilepsy. Epilepsia 56:1124–1133

Ravizza T, Gagliardi B, Noé F, Boer K, Aronica E, Vezzani A (2008) Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. Neurobiol Dis 29:142–160

Ravizza T, Rizzi M, Perego C, Richichi C, Velísková J, Moshé SL, De Simoni MG, Vezzani A (2005) Inflammatory response and glia activation in developing rat hippocampus after status epilepticus. Epilepsia 46(Suppl 5):113–117

Rempe RG, Hartz AMS, Soldner ELB, Sokola BS, Alluri SR, Abner EL, Kryscio RJ, Pekcec A, Schlichtiger J, Bauer B (2018) Matrix metalloproteinase-mediated blood-brain barrier dysfunction in epilepsy. J Neurosci 38:4301–4315

Rizzi M, Perego C, Aliprandi M, Richichi C, Ravizza T, Colella D, Velískŏvá J, Moshé SL, De Simoni MG, Vezzani A (2003) Glia activation and cytokine increase in rat hippocampus by kainic acid-induced status epilepticus during postnatal development. Neurobiol Dis 14:494–503

Robel S, Buckingham SC, Boni JL, Campbell SL, Danbolt NC, Riedemann T, Sutor B, Sontheimer H (2015) Reactive astrogliosis causes the development of spontaneous seizures. J Neurosci 35: 3330–3345

Rogawski MA, Löscher W (2004) The neurobiology of antiepileptic drugs. Nat Rev Neurosci 5: 553–564

Rogove AD, Tsirka SE. Neurotoxic responses by microglia elicited by excitotoxic injury in the mouse hippocampus. Curr Biol. 1998 Jan 1;8(1):19-25. doi: 10.1016/s0960-9822(98) 70016-8. PMID: 9427623.

Roseti C, Fucile S, Lauro C, Martinello K, Bertollini C, Esposito V, Mascia A, Catalano M, Aronica E, Limatola C, Palma E (2013) Fractalkine/CX3CL1 modulates GABAA currents in human temporal lobe epilepsy. Epilepsia 54:1834–1844

Rossini L, Garbelli R, Gnatkovsky V, Didato G, Villani F, Spreafico R, Deleo F, Lo Russo G, Tringali G, Gozzo F, Tassi L, de Curtis M (2017) Seizure activity per se does not induce tissue damage markers in human neocortical focal epilepsy. Ann Neurol 82:331–341

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB (2005) Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 433: 73–77

Sada N, Lee S, Katsu T, Otsuki T, Inoue T (2015) Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. Science 347:1362–1367

Salar S, Maslarova A, Lippmann K, Nichtweiss J, Weissberg I, Sheintuch L, Kunz WS, Shorer Z, Friedman A, Heinemann U (2014) Blood-brain barrier dysfunction can contribute to pharmacoresistance of seizures. Epilepsia 55:1255–1263

Sarac S, Afzal S, Broholm H, Madsen FF, Ploug T, Laursen H (2009) Excitatory amino acid transporters EAAT-1 and EAAT-2 in temporal lobe and hippocampus in intractable temporal lobe epilepsy. APMIS 117:291–301

Sarmast ST, Abdullahi AM, Jahan N (2020) Current classification of seizures and epilepsies: scope, limitations and recommendations for future action. Cureus 12:e10549
Schousboe A, Scafidi S, Bak LK, Waagepetersen HS, McKenna MC (2014) Glutamate metabolism in the brain focusing on astrocytes. Adv Neurobiol 11:13–30
Seifert G, Carmignoto G, Steinhäuser C (2010) Astrocyte dysfunction in epilepsy. Brain Res Rev 63:212–221
Seiffert E, Dreier JP, Ivens S, Bechmann I, Tomkins O, Heinemann U, Friedman A (2004) Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. J Neurosci 24:7829–7836
Sheng J, Liu S, Qin H, Li B, Zhang X (2018) Drug-resistant epilepsy and surgery. Curr Neuropharmacol 16:17–28
Shorvon SD, Goodridge DM (2013) Longitudinal cohort studies of the prognosis of epilepsy: contribution of the National General Practice Study of Epilepsy and other studies. Brain 136: 3497–3510
Simard AR, Rivest S (2004) Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. FASEB J 18:998–1000
Sofroniew MV (2014) Astrogliosis. Cold Spring Harb Perspect Biol 7:a020420
Sontheimer H (2015) Chapter 3—Seizure disorders and epilepsy. In: Sontheimer H (ed) Diseases of the nervous system. Academic, San Diego, pp 61–95
Sosunov AA, Guilfoyle E, Wu X, McKhann GM II, Goldman JE (2013) Phenotypic conversions of "protoplasmic" to "reactive" astrocytes in Alexander disease. J Neurosci 33:7439–7450
Spiciarich MC, von Gaudecker JR, Jurasek L, Clarke DF, Burneo J, Vidaurre J (2019) Global health and epilepsy: update and future directions. Curr Neurol Neurosci Rep 19:30
Stafstrom CE, Carmant L (2015) Seizures and epilepsy: an overview for neuroscientists. Cold Spring Harbor Perspect Med 5(6):a022426
Steinhäuser C, Seifert G, Bedner P (2012) Astrocyte dysfunction in temporal lobe epilepsy: K^+ channels and gap junction coupling. Glia 60:1192–1202
Stergachis AB, Pujol-Giménez J, Gyimesi G, Fuster D, Albano G, Troxler M, Picker J, Rosenberg PA, Bergin A, Peters J, El Achkar CM, Harini C, Manzi S, Rotenberg A, Hediger MA, Rodan LH (2019) Recurrent SLC1A2 variants cause epilepsy via a dominant negative mechanism. Ann Neurol 85:921–926
Streit WJ, Mrak RE, Griffin WS (2004) Microglia and neuroinflammation: a pathological perspective. J Neuroinflammation 1:14
Sun DA, Sombati S, Blair RE, DeLorenzo RJ (2002) Calcium-dependent epileptogenesis in an in vitro model of stroke-induced "epilepsy". Epilepsia 43:1296–1305
Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K (1997) Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 276:1699–1702
Thakran S, Guin D, Singh P, Singh P, Kukal S, Rawat C, Yadav S, Kushwaha SS, Srivastava AK, Hasija Y, Saso L, Ramachandran S, Kukreti R (2020) Genetic landscape of common epilepsies: advancing towards precision in treatment. Int J Mol Sci 21:7784
Tian D-S, Peng J, Murugan M, Feng L-J, Liu J-L, Eyo UB, Zhou L-J, Mogilevsky R, Wang W, Wu L-J (2017) Chemokine CCL2-CCR2 signaling induces neuronal cell death via STAT3 activation and IL-1β production after status epilepticus. J Neurosci 37:7878–7892
Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M (2005) An astrocytic basis of epilepsy. Nat Med 11:973–981
Traynelis SF, Dingledine R (1988) Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. J Neurophysiol 59:259–276
Uhlmann EJ, Wong M, Baldwin RL, Bajenaru ML, Onda H, Kwiatkowski DJ, Yamada K, Gutmann DH (2002) Astrocyte-specific TSC1 conditional knockout mice exhibit abnormal neuronal organization and seizures. Ann Neurol 52:285–296

Ulu MO, Tanriverdi T, Oz B, Biceroglu H, Isler C, Eraslan BS, Ozkara C, Ozyurt E, Uzan M (2010) The expression of astroglial glutamate transporters in patients with focal cortical dysplasia: an immunohistochemical study. Acta Neurochir 152:845–853

Umpierre AD, West PJ, White JA, Wilcox KS (2019) Conditional knock-out of mGluR5 from astrocytes during epilepsy development impairs high-frequency glutamate uptake. J Neurosci 39:727–742

Vajda Z, Pedersen M, Füchtbauer EM, Wertz K, Stødkilde-Jørgensen H, Sulyok E, Dóczi T, Neely JD, Agre P, Frøkiaer J, Nielsen S (2002) Delayed onset of brain edema and mislocalization of aquaporin-4 in dystrophin-null transgenic mice. Proc Natl Acad Sci U S A 99:13131–13136

van der Hel WS, Notenboom RG, Bos IW, van Rijen PC, van Veelen CW, de Graan PN (2005) Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. Neurology 64:326–333

van Vliet EA, Aronica E, Gorter JA (2015) Blood–brain barrier dysfunction, seizures and epilepsy. Semin Cell Dev Biol 38:26–34

van Vliet EA, da Costa Araújo S, Redeker S, van Schaik R, Aronica E, Gorter JA (2007) Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. Brain 130:521–534

Vandenberg RJ, Ryan RM (2013) Mechanisms of glutamate transport. Physiol Rev 93:1621–1657

Vargas-Sánchez K, Mogilevskaya M, Rodríguez-Pérez J, Rubiano MG, Javela JJ, González-Reyes RE (2018) Astroglial role in the pathophysiology of status epilepticus: an overview. Oncotarget 9:26954–26976

Verkhratsky A, Nedergaard M (2018) Physiology of astroglia. Physiol Rev 98:239–389

Verkhratsky A, Parpura V, Pekna M, Pekny M, Sofroniew M (2014) Glia in the pathogenesis of neurodegenerative diseases. Biochem Soc Trans 42:1291–1301

Vezzani A, Aronica E, Mazarati A, Pittman QJ (2013a) Epilepsy and brain inflammation. Exp Neurol 244:11–21

Vezzani A, Bartfai T, Bianchi M, Rossetti C, French J (2011a) Therapeutic potential of new antiinflammatory drugs. Epilepsia 52(Suppl 8):67–69

Vezzani A, French J, Bartfai T, Baram TZ (2011b) The role of inflammation in epilepsy. Nat Rev Neurol 7:31–40

Vezzani A, Friedman A (2011) Brain inflammation as a biomarker in epilepsy. Biomark Med 5: 607–614

Vezzani A, Friedman A, Dingledine RJ (2013b) The role of inflammation in epileptogenesis. Neuropharmacology 69:16–24

Vezzani A, Maroso M, Balosso S, Sanchez MA, Bartfai T (2011c) IL-1 receptor/Toll-like receptor signaling in infection, inflammation, stress and neurodegeneration couples hyperexcitability and seizures. Brain Behav Immun 25:1281–1289

Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, De Simoni MG, Sperk G, Andell-Jonsson S, Lundkvist J, Iverfeldt K, Bartfai T (2000) Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. Proc Natl Acad Sci U S A 97:11534–11539

Vezzani A, Viviani B (2015) Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. Neuropharmacology 96:70–82

Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 6:626–640

Voss LJ, Jacobson G, Sleigh JW, Steyn-Ross A, Steyn-Ross M (2009) Excitatory effects of gap junction blockers on cerebral cortex seizure-like activity in rats and mice. Epilepsia 50:1971–1978

Walker L, Sills GJ (2012) Inflammation and epilepsy: the foundations for a new therapeutic approach in epilepsy? Epilepsy Curr 12:8–12

Wallraff A, Köhling R, Heinemann U, Theis M, Willecke K, Steinhäuser C (2006) The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. J Neurosci 26: 5438–5447

Wang DD et al (2012) Minocycline- and tetracycline-class antibiotics are protective against partial seizures in vivo. Epilepsy Behav 24(3):314–318

Weissberg I, Wood L, Kamintsky L, Vazquez O, Milikovsky DZ, Alexander A, Oppenheim H, Ardizzone C, Becker A, Frigerio F, Vezzani A, Buckwalter MS, Huguenard JR, Friedman A, Kaufer D (2015) Albumin induces excitatory synaptogenesis through astrocytic TGF-β/ALK5 signaling in a model of acquired epilepsy following blood-brain barrier dysfunction. Neurobiol Dis 78:115–125

Woodcock T, Morganti-Kossmann MC (2013) The role of markers of inflammation in traumatic brain injury. Front Neurol 4:18

Wu Y, Wang X, Mo X, Xi Z, Xiao F, Li J, Zhu X, Luan G, Wang Y, Li Y, Zhang J (2008) Expression of monocyte chemoattractant protein-1 in brain tissue of patients with intractable epilepsy. Clin Neuropathol 27(2):55–63. https://doi.org/10.5414/npp27055. PMID: 18402383

Wu XL, Tang YC, Lu QY, Xiao XL, Song TB, Tang FR (2015) Astrocytic Cx 43 and Cx 40 in the mouse hippocampus during and after pilocarpine-induced status epilepticus. Exp Brain Res 233: 1529–1539

Xu Y, Zeng K, Han Y, Wang L, Chen D, Xi Z, Wang H, Wang X, Chen G (2012) Altered expression of CX3CL1 in patients with epilepsy and in a rat model. Am J Pathol 180:1950–1962

Yang Y, Estrada EY, Thompson JF, Liu W, Rosenberg GA (2007) Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. J Cereb Blood Flow Metab 27:697–709

Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA (2006) Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. Nat Neurosci 9: 1142–1149

Zhang JM, Wang HK, Ye CQ, Ge W, Chen Y, Jiang ZL, Wu CP, Poo MM, Duan S (2003) ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. Neuron 40:971–982

Zhang Y, Dong H, Duan L, Yuan G, Liang W, Li Q, Zhang X, Pan Y (2019) SLC1A2 mediates refractory temporal lobe epilepsy with an initial precipitating injury by targeting the glutamatergic synapse pathway. IUBMB Life 71:213–222

Minocycline- and tetracycline-class antibiotics are protective against partial seizures in vivoWang, Doris D. et al.Epilepsy & Behavior, Volume 24, Issue 3, 314 - 318

Tumors of the Glia: Recent Advances

Vaishali Suri, Swati Mahajan, Jyotsna Singh, Iman Dandapath, Rituparna Chakraborty, Afreen Khan, Mehar C. Sharma, and Chitra Sarkar

Abstract

Gliomas are the most common primary malignant central nervous system (CNS) tumors and are associated with significant morbidity and mortality. They can be either diffuse or circumscribed. Diffuse gliomas occur at all ages, though more common among adults. Morphologically they are classified as astrocytomas and oligodendrogliomas, and their grade of malignancy is represented across WHO grades 2–4. Circumscribed gliomas are more frequent in children, the most common type being pilocytic astrocytoma. Recent research has elucidated molecular heterogeneity among glial tumors, which have led to identification of various important genetic and epigenetic pathways that drive glioma initiation and proliferation. Thus astrocytomas are characterized by IDH1/2 mutations, while oligodendrogliomas have 1p/19q co-deletion in addition to IDH1/2 mutations. These molecular alterations also serve as diagnostic and prognostic markers, while *MGMT* promoter methylation is a predictive biomarker. Pediatric-type diffuse gliomas (low grade and high grade) share similar histology with their adult counterparts; however, they harbor distinct genetic alterations. Low-grade diffuse gliomas and circumscribed gliomas are characterized by alterations in the *RAS/MAPK* pathway and high-grade diffuse gliomas by histone H3 gene mutations (H3K27M and H3G34V/R mutations). Numerous clinical trials are ongoing using these markers for targeted therapy in pediatric gliomas. Thus the latest Fifth edition of WHO CNS tumor classification emphasizes on significance of combining histologic and molecular parameters for the integrated diagnosis of brain tumors that would provide valuable diagnostic, prognostic, and predictive information and for some entities, suggest targeted therapies.

V. Suri · S. Mahajan · J. Singh · I. Dandapath · R. Chakraborty · A. Khan · M. C. Sharma · C. Sarkar (✉)
Department of Pathology, All India Institute of Medical Sciences, New Delhi, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_13

Keywords

Gliomas · Adult-type diffuse gliomas · Pediatric-type diffuse gliomas · Circumscribed gliomas · Molecular alterations

1 Introduction

Gliomas are the most common primary central nervous system (CNS) tumors (Coons et al. 1997) accounting for about 30% of all CNS tumors and 80% of all malignant brain tumors (Goodenberger and Jenkins 2012). These neoplasms occur in all ages and at all locations throughout the neuro axis. Research in transgenic mice demonstrated that gliomas could have originated from a gamut of cell types that includes astrocytes, oligodendroglial progenitor cells, or neural stem cells (Parsons et al. 2008).

The classification and grading of gliomas have evolved over time, beginning in 1926 with a system devised by Bailey and Cushing (1926) and later revised by Kernohan, Ringertz, and others (Kernohan and Mabon 1949; Ringertz 1950). Current classification of gliomas is based on the World Health Organization (WHO) classification of CNS tumors, which was first published in 1979 and has been revised four times since then, the most recent being in 2021 (Reifenberger et al. 2017; Louis et al. 2016, 2021a).

Up to 2007 (fourth edition), the WHO classification and grading of gliomas has been based on their histological characteristics, supplemented with immunohistochemistry (IHC) for tumor markers and proliferation index (Santosh 2012). In addition to histological tumor typing, each tumor has been assigned a histological grade based on the degree of anaplasia, from WHO grade I to IV with WHO grade I designating an indolent lesion often associated with a favorable outcome and WHO grade IV designating a malignant tumor associated with poor prognosis (Reifenberger et al. 2017; Louis et al. 2016). Thus for past many decades histomorphological classification has served as the benchmark for glioma diagnostics and therapeutic decision-making. However it is associated with considerable interobserver variability and also does not provide insights into the underlying tumor biology (Pietrak et al. 2011). Therefore it cannot be relied upon completely for tailored treatment of individual patients.

Over the last few decades, genome-wide molecular profiling studies have unfolded the distinct genetic alterations and epigenetic profiles associated with various gliomas. It has also been extensively demonstrated that these molecular characteristics can be exploited as diagnostic, prognostic, therapeutic, and predictive biomarkers and thus can help in refining glioma classification. Thus, the WHO classification of tumors of the CNS has been revised in 2016 (updated fourth edition) to incorporate for the first time molecular biomarkers along with classic histological features in an "integrated diagnosis" format, in an attempt to define distinct glioma entities. The 2016 WHO classification of tumors of the CNS reflects a revolutionary change, replacing conventional histology-based glioma diagnostics with an

integrated histological and molecular classification system that empowers more meticulous tumor categorization (Louis et al. 2016).

However, while the WHO 2016 revision was being prepared, it became apparent that future changes would very soon be needed because of rapid advances in molecular insights of CNS tumors and their impact on diagnosis, prognosis, management, and targeted therapy as well as discovery of novel distinct entities, promising new biomarkers and newer drug targets. Accordingly, the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (c-IMPACT-NOW) was constituted by International Society of Neuropathology (ISN) as a platform to evaluate the recent developments and provide recommendations for proffered changes to succeeding CNS tumor classifications. A total of seven c-IMPACT updates have been published till date which have facilitated the preparation of the Fifth edition of WHO Classification of CNS Tumors (2021) (Louis et al. 2021a).

The Salient Changes in WHO 2021 Classification of CNS Tumors Fifth edition Is as Follows (Louis et al. 2021a)

1. Change of grading to Arabic from Roman numerals (Grade 1 to 4 instead of Grade I to IV).
2. Restructuring of tumor groups, e.g., separation of diffuse and circumscribed gliomas.
3. For the first time, separation of pediatric gliomas as distinct from adult gliomas and their further categorization into diffuse low- and high-grade pediatric type gliomas.
4. Revised nomenclature of some existing entities, e.g.,
   (a) Astrocytoma, IDH mutant, covering grades 2–4 (eliminates the term "anaplastic astrocytoma" and "glioblastoma, IDH-mutant").
   (b) Diffuse midline glioma, H3K27M altered (changes "mutant" to "altered" given multiple mechanisms).
   (c) Astroblastoma, *MN1* altered (adds genetic modifier).
   (d) Chordoid glioma (removes site designation).
5. Addition of newly recognized entities, some on the basis of distinct genetic signatures, e.g.,
   (a) Diffuse astrocytoma, *MYB* or *MYBL1* altered.
   (b) Diffuse low-grade glioma, MAPK pathway altered.
   (c) Diffuse hemispheric glioma, H3 G34 mutant.
   (d) Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype.
   (e) Infant-type hemispheric glioma.
   (f) High-grade astrocytoma with piloid features.
   (g) CNS tumor with *BCOR* internal tandem duplication.

It is hoped that this revolutionary change will make a considerable difference in how glioma is diagnosed and hence have remarkable significance for future clinical trials and patient management in daily practice.

2 Adult-Type Diffuse Gliomas

2.1 WHO 2021 Classification of Adult-Type Diffuse Gliomas

Tumor type	WHO grade
Astrocytoma, IDH mutant	2/3/4
Oligodendroglioma, IDH mutant and 1p/19q co-deleted	2/3
Glioblastoma, IDH wild type	4

2.2 Clinical-Pathological Characteristics of Adult-Type Diffuse Gliomas

Astrocytoma

These tumors can occur in any part of the CNS including the brain stem and the spinal cord. They occur more frequently in the supratentorial than the infratentorial compartment. The majority of grade 2 and grade 3 patients are in the age group of 30–40 years, while patients of grade 4 are slightly older.

Astrocytoma, IDH-Mutant, WHO Grade 2

"A diffusely infiltrative astrocytic glioma with an IDH1 or IDH2 mutation that is well differentiated and lacks histologic features of anaplasia. Mitotic activity is not detected or low. Microvascular proliferation, necrosis and *CDKN2A*/B homozygous deletions are absent" (Louis et al. 2021a). These are relatively slow-growing tumors with a median survival of 7–10 years.

Astrocytoma, IDH-Mutant, WHO Grade 3

"A diffusely infiltrative astrocytic glioma with an IDH1 or IDH2 mutation that exhibits focal or dispersed anaplasia and displays significant mitotic activity. Microvascular proliferation, necrosis and *CDKN2A*/B homozygous deletions are absent" (Louis et al. 2021a). The median survival is approximately 3.5 years.

Astrocytoma, IDH-Mutant, WHO Grade 4

"A diffusely infiltrative astrocytic glioma with an IDH1 or IDH2 mutation that exhibits microvascular proliferation or necrosis or *CDKN2A/B* homozygous deletion or any combination of these features" (Louis et al. 2021a). Though prognosis is poor, it is better than that of GB, IDH wild type.

Oligodendroglioma (ODG)

These tumors occur mainly in the cerebral hemispheres, predominantly in the frontal lobe. Most of the patients are adult with the median age being 45 years.

Oligodendroglioma, IDH-Mutant, and 1p/19q Co-deleted, WHO Grade 2

"A diffusely infiltrative glioma with an IDH1 codon 132 or IDH2 codon 172 missense mutation and combined whole arm deletions of 1p/19q and which lacks histologic features of anaplasia" (Louis et al. 2021a). Median survival is generally >15 years.

Oligodendroglioma, IDH-Mutant, and 1p/19q Co-deleted, WHO Grade 3

"A diffusely infiltrative glioma with an IDH1 codon 132 or IDH2 codon 172 missense mutation and combined whole arm deletions of 1p/19q and histologic features of anaplasia including brisk mitotic activity and/or pathological microvascular proliferation with or without necrosis" (Louis et al. 2021a). This group of tumors does generally well with median survivals reported to be more than 10 years with postoperative adjuvant chemoradiation.

Glioblastoma (GB)

These tumors mainly affect the cerebral hemispheres. They preferentially affect older adults (55–85 years) and comprise 45–50% of all primary malignant brain tumors. Interestingly the term "multiforme" has been removed in the 2021 classification.

Essential Diagnostic Criteria for Glioblastoma, IDH-Wild Type (Louis et al. 2021a)

An IDH-wildtype diffuse astrocytic glioma with:

- microvascular proliferation, or
- necrosis, or
- one or more of the following molecular features GB
- *TERT* promoter mutation, or
- *EGFR* gene amplification, or
- +7/−10 chromosome copy number changes

IDH-wild type GB, also known as primary GB, is the most aggressive of all diffuse gliomas with a median survival of only 12–18 months. The recommended treatment in IDH wild-type GB is maximal safe surgical resection succeeded by radiotherapy with 59.4 Gy/33 fractions and chemotherapy with temozolomide (TMZ), followed by cyclical TMZ, unless contraindicated (Santosh et al. 2019).

Oligoastrocytic Gliomas

In the 2016 and 2021 WHO classification, oligoastrocytomas are no more regarded as distinct tumor type because they have a dearth of distinctive molecular profile and rather have either astrocytic or oligodendroglial genotypes (Yan et al. 2009; Karsy et al. 2017); thus, screening for IDH mutation and 1p/19q co-deletion is required (Louis et al. 2016).

2.3 Key Molecular Alterations in Adult-Type Diffuse Gliomas (Table 1)

Mutations of Isocitrate Dehydrogenase 1 and 2 (*IDH1/2*) Genes

In humans IDH gene comprises of three types of isoenzymes, namely, *IDH1*, *IDH2*, and *IDH3*, which are engaged in the tricarboxylic acid (TCA)/Krebs cycle. While *IDH1* is located predominantly in the cytoplasm and peroxisomes, *IDH2* and *IDH3* are confined to the mitochondria (Kloosterhof et al. 2011). The primary function of *IDH1* and *IDH2* enzymes is to catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) while reducing NADP+ to nicotinamide adenine dinucleotide phosphate (NADPH) in TCA cycle (Huang et al. 2019; Kloosterhof et al. 2011).

Parsons et al. (2008) in a genome-wide sequencing study first reported *IDH1* gene (2q.33) mutations in 12% of GBs (Parsons et al. 2008). Subsequently substantial number of studies authenticated the presence of *IDH1* and *IDH2* mutations in a significant proportion of low-grade gliomas and secondary GBs (Cohen et al. 2013). *IDH1* mutations are specific to gliomas and do not occur in other tumor types of both CNS and non-CNS origin, with notable exceptions being acute myeloid leukemia, angioimmunoblastic T cell lymphoma, and rarely in chondrosarcoma, intrahepatic cholangiocarcinoma, B-acute lymphoblastic leukemia, melanoma, esophageal

Table 1 Common molecular alterations in adult-type diffuse gliomas (WHO 2021)

Molecular characteristic	Astrocytoma	Oligodendroglioma	Glioblastoma	Significance
IDH1/2 mutation	+	+	−	Diagnostic, prognostic, therapeutic target
ATRX nuclear expression	Lost (≥75%)	Retained	Retained	Diagnostic
P53 nuclear expression	+	−	−	Diagnostic
1p/19q whole arm co-deletion	−	+	−	Diagnostic, prognostic, predictive
CDKN2A/B homozygous deletion	+/−	−	+/−	Diagnostic of grade 4 IDH-mutant astrocytoma, prognostic
TERT promoter mutation	+/−	+	+/−	Prognostic, predictive
EGFR gene amplification	−	−	+/−	Diagnostic, therapeutic target
+7/−10 copy number changes	−	−	+/−	Diagnostic

cancer, colorectal cancer, prostate carcinoma, and breast adenocarcinoma (Tateishi and Yamamoto 2019).

Greater than 90% of IDH mutations are located at codon R132 of the *IDH1* gene—substitution of Arg132 with histidine (R132H) (Cohen et al. 2013). The remaining *IDH1* mutations involve substitution of arginine with cysteine R132C (4.7%), glycine R132G (2.1%), serine R132S (1.7%), or leucine R132K (0.8%) (Balss et al. 2008; Yan et al. 2009). *IDH2* mutations are rare (5%) and involve replacement of arginine by lysine at codon 172 (R172K) (Cohen et al. 2013). These IDH mutations are somatic, missense, and heterozygous in nature (Kloosterhof et al. 2011; Jha et al. 2011a).

The mutant *IDH1* carries out a disparate enzymatic reaction involving reduction of α-KG to D-2 hydroxyglutarate (2-HG) coupled with oxidation of NADPH to NADP (Dang et al. 2009; Yen et al. 2010). This 2-HG acts as an oncometabolite and induces DNA hypermethylation by inhibiting a variety of α-KG-dependent dioxygenases such as ten-eleven translocation (TET) family of 5-methyl cytosine hydroxylases. It also promotes global histone methylation by inhibiting histone demethylases such as Jumonji-C domain containing histone-lysine demethylases. In addition to above epigenetic alterations, 2-HG also causes metabolic alterations such as alteration of HIF-1α (hypoxia-inducible factor 1α) activity, downregulation of DNA damaging pathways, etc. (Yen et al. 2010). As a consequence, IDH mutation eventually leads to widespread CpG islands hypermethylation in the promoter region of various genes, a phenomenon termed as glioma CpG island methylator phenotype (G-CIMP). Both DNA and histone hypermethylation possibly arrest cellular differentiation by transcriptional silencing of a wide range of target genes. There is evidence suggesting that IDH mutation is an early happening in the development of gliomas and indeed is the driving force behind the formation of IDH mutant gliomas (Cohen et al. 2013). However, studies in mice implicate that IDH mutation alone is not adequate for tumorigenesis. Indeed, IDH-mutant astrocytomas frequently carry additional alterations in *TP53* and *ATRX* genes, connoting that the development of IDH-mutant astrocytoma requisite numerous genetic "hits."

IDH mutation is seen in oligodendrogliomas and WHO grade 2–4 astrocytomas and thus serves as an important diagnostic biomarker (Table 1) (Cohen et al. 2013; Balss et al. 2008; Jha et al. 2011a). IDH is also a prognostic marker as IDH mutant gliomas have a favorable outcome than their wild-type counterparts regardless of histological grade (Cohen et al. 2013). IDH has also shown potential for targeted therapy. Clinical trials using several small molecule *IDH1*/*IDH2* inhibitors (AG-120, AG-221, AG-881, BAY1436032, and DS-1001b) are being tried out as new therapeutic options to improve patient overall survival (OS) (Karpel-Massler et al. 2019). IDH is also a potential target of single peptide vaccination approaches, which is under clinical trials (Schumacher et al. 2014). IDH mutation thus plays a crucial role in the clinical decision-making, treatment, and prognostication of patients with glioma.

IHC for *IDH1* (R132H) mutant protein has 100% accordance with *IDH1* (R132H) sequencing and is widely accepted method across the world (Agarwal et al. 2013). However, other *IDH1* and *IDH*2 mutations can be tested by only DNA

sequencing. Thus, all cases of diffuse glioma should be initially screened by IHC for *IDH1* (R132H), and only those that are immunonegative should be subjected to DNA sequencing.

Co-deletion of Chromosomes 1p and 19q

Oligodendrogliomas (ODGs) represent the first CNS neoplasm in which a genetic signature has been associated with improved survival. In 1994, Reifenberger et al. demonstrated allelic loss of 19q chromosome in 81% of the oligodendroglial tumors, and of these, approximately 75% also exhibited LOH at 1p locus (Reifenberger et al. 1994). Later, multiple studies confirmed unbalanced translocation between the short arm of chromosome 1 and long arm of chromosome 19 consequential resulting in loss of the derivative chromosome, del (1;19) (p10;q10) in ODGs (Yip et al. 2012; Jha et al. 2010a). Co-deletion of these chromosome arms is a specific molecular alteration, now considered as the essential diagnostic marker of ODG. According to 2016 and 2021 WHO classification, a tumor cannot be labeled ODG if it does not have IDH mutation and 1p/19q co-deletion (Table 1) (Louis et al. 2016, 2021a). This alteration is associated with better OS (prognostic biomarker) and also attributes as an indicator of sensitivity to chemotherapy (predictive biomarker) (Cairncross et al. 1998; Smith et al. 2000; Ino et al. 2001). Long-term follow-up study of phase III clinical trials in patients with anaplastic glioma has revealed that 1p/19q co-deletion is a predictive biomarker of benefit from polychemotherapy with procarbazine, lomustine (CCNU), and vincristine (PCV) if administered immediately before or after radiotherapy (van den Bent et al. 2013). Whether the PCV chemotherapy can be effectively replaced by TMZ will be investigated in the new CO DEL trial (Jaeckle et al. 2021). On the other hand, provisional results from the CATNON trial of therapy with concomitant and adjuvant temozolomide for 1p/19q non-co-deleted anaplastic glioma showed significant survival benefit in patients (van den Bent et al. 2017). Eminently, only whole-arm 1p/19q co-deletion in concurrence with IDH mutation is prognostically favorable; partial deletions on either chromosome arm, which can exist in those with IDH wild-type GBs, result in poor patient outcomes (van den Bent et al. 2013; Mizoguchi et al. 2012).

The genes on 1p and 19q that are considered to be accountable for the effect of 1p/19q co-deletion are FUBP1 and CIC, respectively. Mutations in FUBP1 (encoding far upstream element-binding protein 1, which is responsible for regulating MYC expression) are detectable in approximately one-third of oligodendroglial tumors. CIC mutation (leading to inactivation of the protein homologue of *Drosophila* capicua, a transcriptional repressor) is discernable in more than two-thirds of patients (Bettegowda et al. 2011).

Polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH) are the two most commonly used techniques for the assessment of LOH 1p/19q (Shukla et al. 2009). FISH assay is preferred in most clinical laboratories as it is pathologist friendly and can be performed on formalin-fixed paraffin-embedded (FFPE) tissue sections (Jha et al. 2011b).

Mutation of Alpha Thalassaemia Mental Retardation, X Linked (*ATRX*) Gene

The *ATRX* gene located on chromosome Xq210.1 is a member of the SWI2/SNF2 family of DNA helicases. It is required for histone regulation, nucleosome assembly, chromatin remodeling, and in maintenance of telomeres. A foremost role of *ATRX* is incorporation of histone H3.3 monomers into chromatin in collaboration with the histone chaperone protein DAXX (death-associated protein 6) (Haase et al. 2018, 2020). *ATRX* is involved with the telomerase-independent ALT (alternative lengthening of telomeres) mechanism, the method responsible for telomere repair in most gliomas as well as accountable for DNA damage and replicative stress (Malmstrom et al. 2012; van den Bent et al. 2013).

Mutations in this gene lead to loss of function. *ATRX* mutations occur in $\geq$75% of IDH-mutant grade 2–4 astrocytomas (Jiao et al. 2012). These mutations are mutually exclusive with 1p/19q co-deletion but are strongly linked with *TP53* mutation (Karsy et al. 2017). The presence of IDH, *ATRX*, and *p53* mutation is a gene signature of astrocytomas, while IDH mutation and 1p/19q co-deletion without *ATRX* mutation is hallmark of ODGs (Table 1) (Ikemura et al. 2016; Venneti and Huse 2015). *ATRX* mutation can be detected either by targeted sequencing or more often by loss of nuclear expression utilizing IHC (Chatterjee et al. 2018; Purkait et al. 2017).

Mutation of *TP53* Gene

Termed as "guardian of genome," *TP53* is one of the most common tumor suppressor genes dysregulated in majority of human cancers including gliomas. *TP53* gene plays a crucial role in modulating the various cellular functions like apoptosis, maintaining genomic stability, inhibition of angiogenesis, and regulation of cell metabolism and tumor environment (Zhang et al. 2018; Olafson et al. 2020). *TP53* mutations correlate strongly with astrocytic morphology and are seen in >50% of grade 2 and 3 astrocytomas, while only a small fraction of ODGs and IDH-wt GBs harbor this alteration (Ohgaki and Kleihues 2005; Nayak et al. 2004). Most *TP53* mutations in glioma are seen in exons 5–8 of the DNA binding domain (Louis 1994; Takami et al. 2015). *TP53* gene mutations are generally mutually exclusive with 1p/19q co-deletion. The presence of *IDH1/2* mutation in combination with loss of *ATRX* and *TP53* is diagnostic of grade 2/3/4 astrocytomas (Gillet et al. 2014; Jha et al. 2011c). Strong nuclear positivity for p53 protein by IHC in a considerable percentage of tumor cells aids as a surrogate marker for *TP53* mutations with a sensitivity and specificity of 77.4–78.8% and 78.6–96.7%, in comparison to DNA sequencing (Takami et al. 2015; Rathore et al. 1999).

Amplification of Epidermal Growth Factor Receptor (*EGFR*) Gene

EGFR gene resides on chromosome 7q12 and serves as receptor tyrosine kinase. *EGFR* activation promotes cellular proliferation via activation of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase-protein kinase B (PI3K-Akt) pathways (Patel and Leung 2012). *EGFR* amplification is considered a

classic genetic and diagnostic marker of IDH wild-type GBs and is found in approximately 40% of these tumors (Bale et al. 2019; Jha et al. 2011a). About half of the *EGFR*-amplified tumors also harbor a genetic rearrangement that leads to deletion of exons 2–7 of EGFR gene resulting in the expression of *EGFR* variant III (*EGFR* vIII). This variant has absence of the extracellular ligand-binding region ciphered by the deleted exons but is constitutively active (Fu et al. 2012; Beiko et al. 2014). The use of *EGFR* amplification and *EGFR*vIII as a prognostic marker is conflicting. Regarding targeted therapy, in spite of the high occurrence of *EGFR* alterations in GBs, these tumors show limited clinical benefit to tyrosine kinase inhibitors (De Witt Hamer 2010). Recently Phase II clinical trials have implicated that vaccination of newly diagnosed GB patients with *EGFR*vIII vaccine (rindopepimut) along with adjuvant granulocyte macrophage colony-stimulating factor (GM-CSF) leads to prolonged recurrence-free and OS with nominal toxicity (Sampson et al. 2010; An et al. 2018).

FISH technique can readily be used to determine *EGFR* amplification (Korshunov et al. 2005, 2019). The presence of the *EGFRvIII* mutation can be assessed by IHC using antibodies to *EGFR* vIII or by PCR sequencing (Aldape et al. 2004).

Mutation in the Promoter Region of Telomerase Reverse Transcriptase (*TERT*) Gene

In the last decade, mutations in the promoter site of the telomerase reverse transcriptase (*TERTp*) gene have been detected in a wide range of tumors (90%), including gliomas (Low and Tergaonkar 2013; Shay and Bacchetti 1997). Telomerase is well known to have a pivotal role in tumorigenesis in various brain tumors, including astrocytomas, GBs, and ODG. Telomerase has two prime components, the 1132-amino acid *TERT* and an affiliated telomerase RNA molecule (TERC). The *TERT* gene is present on the short arm of chromosome 5 (Cong et al. 1999). The two most frequent mutations in the *TERTp* are C228T and C250T, which present −124 base pairs (bp) and −146 bp, upstream of the ATG start site. These two mutations are mutually exclusive and grant unimpeded growth characteristics to tumor cells by expanding telomere length by *TERT* overexpression and subsequently activating telomerase activity, thus contributing to tumorigenesis by overcoming cellular senescence and inducing cell immortalization (Sun et al. 2015; Labussière et al. 2014; Kim et al. 2018; Pekmezci et al. 2017).

*TERT*p mutation is a molecular hallmark of glioma, occurring in 70–80% of primary GBs and about 70–97% of ODGs but less commonly in grade 2 and grade 3 astrocytomas (39%). IDH-mutated and 1p/19q co-deleted ODGs have a higher prevalence of *TERT*p mutations (>90%) compared with IDH-mutated grade 2 (24%) and grade 3 (14%) astrocytomas and IDH wild-type grade 2 (2%) and grade 3 astrocytomas (8%) (Eckel-Passow et al. 2015). This molecular alteration is notably more prevalent in older adults as compared to young adults (61.2% vs. 25%) (Purkait et al. 2016a). Furthermore patients with the concurrent IDH and *TERT*p mutations have better OS (median OS, 246.5 months) than those with IDH-only (median OS, 110.6 months) or *TERT*-only mutations (median OS, 11.6 months) (Heidenreich

et al. 2015; Purkait et al. 2016a). Also patients with both *TERTp* mutation and *EGFR* amplification have poorest OS (mOS 11 months (You et al. 2017; Mosrati et al. 2015)). Recently it is advocated that gliomas with only *TERT* mutations are primarily grade 4 gliomas with an unfavorable course and poor survival. Further the C228T mutation is the more prevalent cancer-associated *TERTp* variant and is associated with poorer OS compared to patients with C250T gliomas (Yuan et al. 2016; Johanns et al. 2016; Kim et al. 2018).

TERT has become an emerging candidate for targeted therapy because of their observed upregulation in a wide variety of cancers, including GBs. Small molecular inhibitor imetelstat (GRN163L), GABPβ1L inhibition, targeting RNA-dependent RNA polymerase (RdRP) activity of *TERT* using eribulin and more recently immunotherapy, is actively being explored as therapeutic target against *TERT* (Jafri et al. 2016; Patel et al. 2020; Li et al. 2020).

Sanger sequencing is considered as a gold standard to identify *TERTp* mutations. Droplet digital PCR-based *TERTp* mutation detection requires a lower tumor DNA quantity and can be used for precise and swift assessment of the *TERTp* mutations (Bai et al. 2021).

Homozygous Deletion of Cyclin-Dependent Kinase Inhibitor 2A/2B (*CDKN2A/2B*) Gene

The *CDKN2A/B* locus is localized on chromosome band 9p21, which via p14ARF and p16INK4A tumor suppressor proteins inhibits the cell cycle of tumor cells (Fontana et al. 2019). The p14ARF causes cell cycle arrest by inhibiting MDM2 and stabilizing p53, thus promoting cell repair and apoptosis phenomena (Serrano et al. 1993). The p16INK4A contributes to cell cycle regulation by inhibiting cyclin-dependent kinases 4 and 6 (CDK4 and CDK6)-mediated phosphorylation of the retinoblastoma pathway leading to G1-phase arrest in the cell cycle (Sherr and Roberts 1999). The most recent theory is that homozygous deletion at 9p21 (*CDKN2A/B* locus) modifies its inhibitory function and promotes unrestricted tumor cell proliferation and increased oncogenic burden.

This genetic alteration has been associated with enhanced oncogenesis and unfavorable prognosis in many tumor types, such as melanoma (Guo et al. 2019), ovarian cancer (Xia et al. 2019), bladder cancer (Worst et al. 2018), and sarcoma (Bui et al. 2019).

Multiple studies have identified that *CDKN2A/B* homozygous deletions are enriched among IDH-mutant astrocytomas that are associated with poor prognosis and that its presence conforms to clinical behavior of WHO grade 4 gliomas. This group also harbored lower levels of global DNA methylation (Aoki et al. 2018; Appay et al. 2019; Cimino et al. 2017; Korshunov et al. 2019; Purkait et al. 2013). The prevalence of *CDKN2A/B* homozygous deletions documented in IDH-mutant astrocytic gliomas ranges from 0% to 12% in WHO grade 2, 6–20% in WHO grade 3, and 16–34% in WHO grade 4 tumors (Appay et al. 2019; Purkait et al. 2013). Homozygous deletion of *CDKN2A/B* is also observed in pediatric low-grade gliomas (6–20%), although at a lesser frequency than in adult glioma (Ryall et al. 2020). Furthermore, *CDKN2A/B* deletion commonly concurs with *BRAF* p.V600E,

implying that it probably acts as a second molecular hit, promoting evasion from cell cycle regulation. FISH assay can directly visualize the status of the genetic locus and reliably distinguish true homozygous deletion from hemizygous loss.

Promoter Methylation of O^6-Methylguanine-DNA Methyltransferase (*MGMT*) Gene

MGMT gene is localized on chromosome 10q26 and encodes for a DNA repair enzyme that provides resistance to alkylating or methylating chemotherapeutic agents by detaching alkyl adducts from O^6 position of guanine, thus ensuring rapid DNA repair (Weller et al. 2010). The standard drug used for GB is temozolomide (TMZ), which is a DNA alkylating agent. Methylation of the promoter region of the *MGMT* gene leads to its epigenetic silencing, thus compromising the DNA repair process and ultimately leads to cell death (Suri et al. 2011a; Hotta et al. 1994).

MGMT promoter methylation status has emerged as one of the critical determinants of prognosis and prediction of response to TMZ in newly diagnosed GBs with methylated *MGMT* promoter being linked with remarkably better outcome and benefit from TMZ as compared to unmethylated phenotype (Stupp et al. 2005; Weller et al. 2010; Wen and Kesari 2008; Mansouri et al. 2019; Hegi et al. 2005; Wick et al. 2012; Purkait et al. 2016a). However assessment of *MGMT* status has not yet been integrated for treatment decision-making in newly diagnosed GBs as current practice does not withhold TMZ from unmethylated patients due to lack of alternative treatment modalities (Mansouri et al. 2019; Butler et al. 2020).

Interestingly, the NOA-08 and Nordic trials documented that in elderly patients, who were administered TMZ alone, *MGMT* promoter methylation was related with notably longer survival than unmethylated tumors (Wick et al. 2012; Malmström et al. 2012). Thus, the 2017 European Association for Neuro-Oncology (EANO) guidelines recommended that *MGMT* testing be contemplated as a standard practice for giving TMZ treatment to malignant gliomas in elderly patients (>65–70 years), while patients with unmethylated tumors should receive hypofractionated radiotherapy alone (Weller et al. 2017).

A wide range of assays is presently available to assess *MGMT* promoter methylation status at DNA, RNA, and protein levels. The most frequently used techniques are methylation-specific PCR (MSP), bisulfite sequencing, real-time quantitative MSP, pyrosequencing, combined bisulfite restriction analysis, methylation-specific multiplex ligation-dependent probe amplification, genome-wide analysis of DNA methylation (EPIC BeadChip Array 450K/850K), and IHC (Preusser 2009; Jha et al. 2010b). Discrepancy in detection methods, optimal cutoff definitions, and standardization among different laboratories represent a key challenge for consensus regarding the best assay (Sauerbrei et al. 2018). However, presently pyrosequencing has evolved as the gold standard as it provides quantitative methylation information up to single base pair resolution and is cost-effective (Mansouri et al. 2019).

3 Pediatric-Type Diffuse Gliomas

Brain tumors are the commonest solid neoplasm of childhood, and 45.7% of childhood brain tumors are glioma (Ostrom et al. 2019). In a multicentric study from India, the most frequent primary brain tumors in pediatric population were astrocytic tumors (34.7%), followed by embryonal tumors (Jain et al. 2011). Though pediatric and adult gliomas appear histologically similar, pediatric gliomas differ considerably from their adult counterparts in terms of prime molecular alterations, infrequency with which the low-grade gliomas progress to higher-grade tumors, and biological behavior (Suri et al. 2009, 2011b; Jha et al. 2015; Pathak et al. 2015; Kumar et al. 2015). Hence they should be treated differently. In the WHO 2021 classification, pediatric gliomas are considered as a separate group for the first time and are further subdivided into low- and high-grade gliomas.

3.1 WHO 2021 Classification of Pediatric-Type Diffuse Gliomas

Category	Subcategory	WHO grade
Pediatric-type diffuse low-grade gliomas	Diffuse astrocytoma, *MYB* or *MYBL1* altered	1
	Angiocentric glioma	1
	Polymorphous low-grade neuroepithelial tumor of the young	1
	Diffuse low-grade glioma, MAPK pathway altered	Grade not yet assigned
Pediatric-type diffuse high-grade gliomas	Diffuse midline glioma, H3K27 altered	4
	Diffuse hemispheric glioma, H3G34 mutant	4
	Diffuse pediatric-type high-grade gliomas, H3wt/*IDH* wt	4
	Diffuse midline glioma, *EGFR* mutant	4
	Infant-type hemispheric glioma	Grade not yet assigned

3.2 Pediatric-Type Diffuse Low-Grade Gliomas (PLGG)

In the past the extent of surgical resection, histological diagnosis, and age were used to ascertain prognosis in pediatric LGG. However currently the molecular underpinnings of pediatric LGG have surfaced as a potent tool to supplement the stratification of these tumors. The data has led to rise in use of targeted therapies that complement and/or replace other cytotoxic approaches.

Diffuse gliomas in children have dearth of both IDH mutation and 1p/19q co-deletion unlike their adult counterparts. Pediatric LGG are predominantly driven by a single genetic event leading to upregulation of the RAS-mitogen-activated

Table 2 Clinicopathological and molecular differences between rearrangement versus SNV-driven PLGG (Ryall et al. 2020)

Characteristics	Rearrangement-driven PLGG	SNV-driven PLGG
Age at diagnosis	Younger age of onset	Older age
	Mean age: 7.6 years	Mean age: 10.1 years
WHO histology	Grade 1	Grade 1 or 2 (2 > 1)
Molecular alteration	Usually one alteration	Often more than one alteration
	KIAA1549-BRAF: 68%	*BRAF* p. V600E: 62%
	Other *BRAF* fusions: 1.5%	Others *BRAF* SNV: 1.6%
	FGFR-TACC1: 5.3%	*FGFR1* SNV: 17%
	FGFR1 TKD: 10%	*IDH1* pR132H: 5.3%
	FGFR2: 3.8%	H3.3p.K27M: 6.4%
	MYB: 5.3%	Others: 8%
	MYBL1: 2.3%	
	Others: 3.8%	
Biological behavior	Less aggressive, indolent even if not completely resected	More aggressive with higher risk of recurrence
	Infrequent recurrence	
Clinical outcome	Good long-term outcome	Relatively poor outcome
	10 year OS: 97.8%	10 year OS: 88.1%

protein kinase (RAS/MAPK) pathway. Majority of these are somatic events involving *BRAF* alterations. Infrequent alterations affecting RAS/MAPK pathway including *FGFR1*/2/3, NTRK2, *RAF1*, *ALK*, and *ROS1* and those affecting non-RAS/MAPK pathway such as *MYB*, *MYBL1*, *IDH1*, and H3F3A have been identified in limited numbers of cases (Ryall et al. 2020).

The genetic alterations in PLGG are generally of rearrangement (fusions) and single nucleotide variation (SNV)/mutations driven as shown below (Table 2).

Key Molecular Alterations in PLGG

The most common alterations in PLGG are in the *BRAF* gene which is an oncogene localized on chromosome 7q34. Two major alterations in the *BRAF* gene include the fusion and the mutation.

KIAA1549-BRAF Fusions

BRAF is a downstream target of the RAF family of serine/threonine protein kinases and a prime signaling molecule of the MAPK pathway, which affects cell division, differentiation, and invasion (Kilday et al. 2014). Initial studies found focal gains on chromosome 7q34, which encoded *BRAF* gene. This gain is the outcome of a 2 Mb tandem duplication at 7q34 leading to the emergence of a novel oncogenic fusion involving *BRAF* and *KIAA1549* (large but uncharacterized gene). This rearrangement results in loss of N-terminal regulatory domain of *BRAF* leading to constitutively activated RAS/MAPK signaling pathway (Jones et al. 2008). Five different *KIAA1549-BRAF* exon-exon combinations have been documented in the following

order of prevalence: 16:9 (49%), 15:9 (35%), 16:11, 18:10, and 19:9, all resulting in the gain of uncontrolled *BRAF* kinase domain (Lin et al. 2012).

KIAA1549-BRAF is the most common genetic alteration in PLGGs (30–40%) and is remarkably enriched in pilocytic astrocytoma (PA) (70%) and predominantly in tumors having origin in the posterior fossa or cerebellum. The *KIAA1549-BRAF* fusion has also been reported rarely in gangliogliomas (GG), pleomorphic xanthoastrocytomas (PXA), diffuse astrocytomas, and pilomyxoid astrocytomas (Zhang et al. 2013; Taha et al. 2015; Kumar et al. 2015; Pathak et al. 2017).

In general detection of *KIAA1549-BRAF* serves as a diagnostic marker for PLGG as it has not been identified in adult-type diffuse gliomas and thus, with rare exception, confirm diagnosis of PLGG (Kumar et al. 2015). Tumors with a *KIAA1549-BRAF* fusion are usually receptive to complete surgical resection and have favorable OS and rarely progress as they have predisposition to arise in highly circumscribed histologies (PA) and in surgically susceptible locations (cerebellum) (Becker et al. 2015; Hawkins et al. 2011; Horbinski et al. 2010; Lassaletta et al. 2016). Thus *KIAA1549-BRAF* can also serve as a prognostic marker.

Further it can also help in identifying tumors perceptible to target therapeutics. Presently, *MEK* inhibitors like selumetinib, trametinib (NCT03363217), cobimetinib (NCT02639546), and binimetinib (NCT02285439) are undergoing clinical trials for PLGGs (*NF1*-pLGG, *KIAA1549-BRAF* fused PLGG) (Ryall et al. 2017).

BRAF V600E Mutation

Missense mutations in *BRAF*, essentially in which valine is substituted by glutamine at amino acid position 600 (p.V600E), act as a phosphomimetic within the RAS/MAPK signaling pathway, perpetuating it constitutively active (Davies et al. 2002). This mutation has been reported in PXA (40–80%), GG (18–33%), dysembryoblastic neuroepithelial tumor (20–25%), PAs (9%), and in rare cases of pediatric low-grade diffuse gliomas. It has also been identified in ~10% of GBs, particularly the epithelioid variant (Gierke et al. 2016; Horbinski et al. 2010; Lassaletta et al. 2016; Schiffman et al. 2010; Schindler et al. 2011; Dodgshun et al. 2016; Kakkar et al. 2016a, 2017).

BRAF V600E-mutant PLGGs are mostly seen in younger age and have poor OS and PFS as opposed to other PLGGs (Chen et al. 2017; Lassaletta et al. 2016). Since *BRAF* is a druggable target, a beneficial response to *BRAF* inhibitors (vemurafenib, dabrafenib) has been demonstrated in *BRAF* V600E-mutant PXAs, brain stem GGs, and pediatric GBs (NCT02124772).

3.3 Pediatric-Type Diffuse High-Grade Gliomas (PHGGs)

PHGGs are histologically and molecularly diverse CNS malignancies that constitute approximately 8% of all pediatric gliomas (Sun et al. 2020). Approximately one-half of PHGGs occur in the brain stem, mostly within the pons as diffuse intrinsic pontine glioma and less frequently in other midline structures. Although they share histological features with that of adult malignant gliomas, in the past few years, the

substantial utilization of genomic profiling techniques has revealed considerable molecular differences between the two age groups. Therefore it became evident that it is important to avoid therapeutic strategies purely using data obtained from studies on adult GBs (Jha et al. 2014, 2015; Pathak et al. 2015). Collaborative genomic analysis further resulted in paramount reclassification of PHGG depending upon molecular alteration with significant clinical correlations with regard to the age at presentation, anatomical location, and prognosis.

Key Molecular Alterations in PHGG

The breakthrough discovery that best exemplifies the distinctive biology of PHGGs was the demonstration of somatic histone mutations. PHGGs are unique in comparison to their adult counterparts, as less than 5% are *IDH1/2* mutated. The same holds true for 1p/19q deletion, which is rarely seen in children. The overall genomic landscape of PHGG includes mutations in histone variants (H3K27M and H3G34R/V) and targetable fusions including *NTRK*, *ALK*, and *ROS* recently detected in infant gliomas. Some of the common molecular genetic alterations are listed below.

H3K27M Mutation

Histones are primary molecules responsible for providing structural support to the chromatin and regulating the gene expression. Histones H2A, H2B, H3, and H4 assemble into an octamer that, via H1, leads to wrapping of DNA around it (Vuorinen et al. 2003). Posttranslational modification of its N-terminal region regulates DNA transcription, replication, and repair. Furthermore, methylation of histone H3 lysine 27 (H3K27) plays a pivotal role in tumorigenesis (Vuorinen et al. 2003; Jha et al. 2014, 2015).

Most of the diffusely infiltrative astrocytomas having origin within the midline structures (thalamus, brain stem, and spinal cord) in pediatric patients are characterized by mutually exclusive somatic driver mutations in H3F3A or HIST1H3B/C. They encode for histone 3 variants H3.3 and H3.1, respectively (Schwartzentruber et al. 2012; Haase et al. 2020; Wu et al. 2012; Pathak et al. 2015). H3K27M mutation results in amino acid replacement of lysine by methionine at position 27 of the histone H3 tail (Karremann et al. 2018). The H3K27M-mutant protein inhibits polycomb repressive complex 2 (PRC2) function by detaching its catalytic subunit enhancer of zeste homolog 2 (EZH2), leading to widespread reduction in trimethylation of H3K27 (H3K27me3) (Bender et al. 2013; Purkait et al. 2015). Extensive gene dysregulation occurs as a result of H3K27M mutation, which includes altered activity of *PDGFRA*, p53, *MYCN*, *ATRX*, *DAXX*, and *ACVR1*.

The fifth edition of the WHO has introduced the term "diffuse midline glioma (DMG), H3K27 altered" for these tumors. They constitute 10–15% of all pediatric brain tumors and classically involve the pons and other midline structures. Diagnostic criteria include midline location, presence of H3K27 mutation, and loss of H3K27me3 by IHC. The overall incidence of H3K27M mutations in DMGs is estimated to be 80% in pediatric patients and is associated with worse OS (median

11–15 months) when compared with wild-type, unrelated to patient age and histological diagnosis. Prognosis of these tumors is poor with 2 years survival rate less than 10%. Therapeutic targeting of histone modifiers has become an area of increased interest in pediatric neuro-oncology (Manjunath et al. 2021).

H3G34 Mutation

Mutations on H3F3A leading to replacement of glycine by arginine or valine at position 34 (G34R/V) have been found in approximately 20% of the PHGG arising in the cerebral hemispheres (Haase et al. 2020; Hakar and Wood 2020). They occur predominantly in the age range of 11–30 years (median 15 years). Patients having tumor with this mutation usually tend to have a longer survival (median survival of 20 months) than DMG (Coleman et al. 2020). A considerable number of H3.3G34R/V-mutated tumors have co-mutations in chaperone genes *ATRX* and *DAXX* and in *TP53* (Sturm et al. 2012).

Histologically, these tumors exhibit two distinct morphologies, "GB-like" and "primitive neuroectodermal tumor (PNET)-like." Strong nuclear immunoexpression of p53 protein and total nuclear immunonegativity for antibodies against *ATRX* protein are noted in >90% of these cases. Although H3G34R/V mutation-specific antibodies have demonstrated high specificity (>95%), infrequently false-negative cases have been recognized recommending being vigilant in their use as surrogate for detection of G34 mutations (Hakar and Wood 2020; Ahrendsen and Alexandrescu 2020; Wood et al. 2019; Haque et al. 2017).

4 Circumscribed Gliomas

Circumscribed gliomas are WHO grade 1 and 2 tumors that occur more commonly in children and young adults. They are slow-growing tumors and are generally curable by resection alone. The most frequent types are pilocytic astrocytoma (PA), pleomorphic xanthoastrocytoma (PXA), and subependymal giant cell astrocytoma (SEGA). The molecular alterations found in adult diffuse gliomas are infrequent in the circumscribed gliomas and, when present, suggest aggressive nature. The cIMPACT-NOW has recognized that there is an overlap between the histologic features and genetic alterations of the pediatric-type diffuse low-grade gliomas and those of circumscribed glial tumors of the childhood. Therefore, they strongly endorsed the advantage of the integrated approach for a range of glial/glioneuronal tumors from clinical point of view (Ellison et al. 2019). The WHO 2021 classification of circumscribed gliomas is as follows (Louis et al. 2021a).

4.1 WHO 2021 Classification of Circumscribed Gliomas

Tumor	WHO grade
Pilocytic astrocytoma	1
High-grade astrocytoma with piloid features	Grade not yet assigned
Pleomorphic xanthoastrocytoma	2 or 3
Subependymal giant cell astrocytoma	1
Chordoid glioma	2
Astroblastoma, *MN1* altered	Grade not yet assigned

4.2 Pilocytic Astrocytoma

PA accounts for about 20% of all childhood primary brain tumors and is the most common glioma in children. The cerebellum is the most common site followed by the optic nerve, optic chiasma/hypothalamus, brain stem, basal ganglia, and spinal cord. Characteristic histopathological features include biphasic loose and compact growth pattern, bipolar hairlike pilocytic cells with presence or absence of rosenthal fibers and/or eosinophilic granular bodies, and low proliferative activity. It is associated with MAPK gene alteration, most often *KIAA1549 BRAF* gene fusion (Louis et al. 2016).

4.3 Pleomorphic Xanthoastrocytoma

PXA typically occurs in pediatric population and young adults with a mean age of 26 years. Majority of the tumors occur supratentorially most frequently in the temporal lobe with superficial location and involving the leptomeninges. Histologically it is characterized by pleomorphic tumor cells including large multinucleated cells, spindled cells, xanthomatous (lipidized) cells, eosinophilic granular bodies, and reticulin deposition. Molecular genetic alteration characteristic of PXA is *BRAF* V600E mutation and homozygous *CDKN2A/B* deletion (Louis et al. 2016). *TERTp* mutation is present especially in anaplastic/grade 3 PXA.

4.4 Subependymal Giant Cell Astrocytoma

SEGA usually occurs during first two decades of life and is often associated with tuberous sclerosis complex. They typically arise from the lateral ventricle adjacent to the foramen of monro and infrequently in the third ventricle. Characteristic histological features include multiple polygonal, gemistocyte-like cells, and ganglionic-like cells showing immunoreactivity for the glial markers and variable immunoexpression of neuronal markers (Sharma et al. 2004; Kumari et al. 2016; Louis et al. 2016).

5 Conclusion

Progress in genomics along with significant advances in immunology have defined a new molecular era in neuro-oncology. The identification of driver alterations in gliomas has highlighted their biological and clinicopathological heterogeneity and opened new diagnostic and therapeutic avenues. Keeping pace with recent advances, molecular markers have been incorporated as diagnostic, prognostic, and therapeutic indicators in the updated WHO 2021 classification. It is anticipated that such modifications will provide pragmatic guidelines to pathologists and neuro-oncologists worldwide and will be beneficial to the patients who are affected by CNS tumors. Useful and meaningful data can be acquired with careful histomorphological analysis, immunohistochemical evaluation, FISH assay, and Sanger sequencing technologies, which are available at most centers and together can provide comprehensive and integrated diagnosis of majority of gliomas. This is vital for stratification of patients in future clinical trials to develop new targeted therapies for these tumors. Overall molecular information now manifests a prerequisite part of neuropathology diagnosis, and it is expected that the range of molecular testing techniques will grow and evolve over time.

Acknowledgement None.

Funding: None.

Annexure I: Contributions from Neuropathology Unit, Department of Pathology, AIIMS, New Delhi

Significant research contributions have been made in pediatric and adult neuro-oncology. The knowledge of medical science has been combined with insight into molecular biology and genetics to obtain novel insights into the biology of gliomas. Some of the work has been taken from bench to bedside, and the role of various immunohistochemical, proliferation, and molecular markers has been established for more accurate objectivized diagnosis, prognostication, and predicting treatment response of gliomas. Many of these biomarkers are now used in routine neuropathology practice to supplement histopathological classification and grading for better patient management.

1. Initial studies have established the light microscopic and ultrastructural morphological diversity and histogenesis of various glial tumors with documentation of several novel and rare phenotypes/variants. Studies on expression of immunohistochemical tumor markers have established their role as diagnostic biomarkers (Sarkar et al. 1988, 1997, 2005; Roy et al. 1988; Dinda et al. 1990, 1992; Sharma et al. 1996, 2004, 2006; Karak et al. 2000; Srivastava et al. 2004; Deb et al. 2005, 2006; Malik et al. 2006; Agarwal et al. 2012; Khanna et al. 2018).

2. Studies related to in vivo and in vitro cell proliferation kinetics, apoptosis, and angiogenesis have contributed significantly to understanding the biological aggressiveness of gliomas and their role as prognostic biomarkers (Kharbanda et al. 1993, 1995; Dinda et al. 1993a, b; Banerjee et al. 1996; Rathore et al. 1999; Ralte et al. 2001; Sharma et al. 2004, 2006; Sarkar et al. 2005; Avninder et al. 2006; Das et al. 2011).
3. Studies in molecular neuro-oncology have given novel insights into the genetic and epigenetic events fundamental to glioma initiation and progression. Studies also identified specific molecular alterations that serve as diagnostic, prognostic, and predictive biomarkers (Banerjee et al. 1996; Chattopadhyay et al. 1997; Rathore et al. 1999; Misra et al. 2000; Sarkar et al. 2000, 2002, 2003, 2004; Sharma et al. 2004, 2006; Nayak et al. 2004; Srivastava et al. 2004; Avninder et al. 2006; Shukla et al. 2009; Jha et al. 2010a, b, 2011a, c, 2015; Kakkar et al. 2011, 2016a, b; Das et al. 2011; Agarwal et al. 2013; Purkait et al. 2013, 2015, 2016a, b).
4. Studies have provided better understanding of genetic heterogeneity within tumors of the same histological type and grade, thus explaining varying tumor behavior. Simple, economical, and reliable prognostic signatures/risk stratification systems based on both genetic and epigenetic markers have been developed, which can separate histologically similar tumors of the same grade into prognostically relevant subgroup, thus paving the way for personalized medicine (Purkait et al. 2016a, b, c).
5. New insights have been gained into epigenetic regulation in gliomas. Studies on polycomb repressive complexes have highlighted the role of the epigenetic regulator EZH2 (enhancer of zest homologue 2) and its positive correlation with DNA methyl transferases (DNMT1 and DNMT3B) and microRNA network in GBMs (Purkait et al. 2015, 2016b; Sharma et al. 2016). Further, studies on EZH2 and trimethylation of histone H3 on lysine 27 (H3K27me3) using whole-genome ChipSeq analysis highlight the differences in genes and pathways targeted by H3K27me3 in GBMs vs. low-grade gliomas. This work has produced the first high-resolution genome-wide map of H3K27me3 modification in adult human primary glioma samples. Interestingly, SLC25A23, a calcium-dependent mitochondrial solute carrier gene and an important target of H3K27ME3 modification, was identified as a potential new prognostic biomarker in GBMs, which needs further validation (Sharma et al. 2017).
6. Recent studies on the role of micro-RNA clusters have demonstrated tumor-suppressive role of C14MC in oligodendrogliomas and glioblastomas (Kumar et al. 2018; Nayak et al. 2018). Further, two specific microRNAs with potential therapeutic values have been identified in GBMs, which may be relevant for development of new therapeutic strategies. Also, for the first time, it has been demonstrated that the calcitonin-calcitonin receptor (CT-CALCR) axis is an important tumor suppressor pathway in gliomas, and mutations in the receptor predict poor prognosis. Thus, the CALCR could be considered as a novel therapeutic target for GBM (Pal et al. 2018).

7. Work in pediatric neuro-oncology is indeed novel as it has established that pediatric tumors, though histomorphologically indistinguishable from their adult counterparts, are a distinct molecular entity both genetically and epigenetically. Thus, it has been shown that pediatric GBMs are distinctly different from adult GBMs in terms of genetic alterations, histone methylation, whole-genome DNA methylation profile, and genome-wide small noncoding RNA profile. Similar molecular genetic differences have been demonstrated between pediatric and adult oligodendrogliomas and pilocytic astrocytomas. For the first time, the possible role of reactive oxygen species, altered global histone methylation, and downregulation of snoRNA cluster HbII-52 has been established as novel mechanisms of pediatric GBM pathogenesis. The first genome-wide profiling study of noncoding RNA in pediatric GBMs has highlighted the downregulation of Sno-RNA which have now been shown to be upcoming drivers of cancer. Findings indicate that results from adult cases cannot simply be extrapolated to pediatric patients, thus highlighting the need for identification of separate prognostic markers and molecular targeted therapy tailored for age (Suri et al. 2009, 2011b; Jha et al. 2011d, 2014, 2015, 2019; Purkait et al. 2013; Kumar et al. 2015; Pathak et al. 2015; Kakkar et al. 2016a, 2017; Purkait et al. 2017; Agrawal et al. 2018; Santosh et al. 2019; Manjunath et al. 2021).
8. This unit faculty has contributed as coauthors to chapters in successive editions (2000, 2007, 2016 and 2021) of the WHO Classification of CNS Tumors (Becker et al. 2000; McLendon et al. 2007, 2016; Lopes et al. 2007, 2016, 2021; Korshunov et al. 2016; Louis et al. 2020, 2021b; Brat et al. 2020; Brandner et al. 2021; Ellison et al. 2021). About a dozen publications of this unit are cited in the reference list of the WHO fascicles.

References

Agarwal S, Sharma MC, Singh G, Suri V, Sarkar C, Garg A, Kumar R, Chandra PS (2012) Papillary glioneuronal tumor—a rare entity: report of four cases and brief review of literature. Childs Nerv Syst 28:1897–1904

Agarwal S, Sharma MC, Jha P, Pathak P, Suri V, Sarkar C, Chosdol K, Suri A, Kale SS, Mahapatra AK, Jha P (2013) Comparative study of IDH1 mutations in gliomas by immunohistochemistry and DNA sequencing. Neuro-Oncology 15:718–726

Agrawal R, Garg A, Benny Malgulwar P, Sharma V, Sarkar C, Kulshreshtha R (2018) p53 and miR-210 regulated NeuroD2, a neuronal basic helix-loop-helix transcription factor, is downregulated in glioblastoma patients and functions as a tumor suppressor under hypoxic microenvironment. Int J Cancer 142:1817–1828

Ahrendsen J, Alexandrescu S (2020) An update on pediatric gliomas. Surg Pathol Clin 13:217–233

Aldape KD, Ballman K, Furth A, Buckner JC, Giannini C, Burger PC, Scheithauer BW, Jenkins RB, James CD (2004) Immunohistochemical detection of EGFRvIII in high malignancy grade astrocytomas and evaluation of prognostic significance. J Neuropathol Exp Neurol 63:700–707

An Z, Aksoy O, Zheng T, Fan QW, Weiss WA (2018) Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies. Oncogene 37:1561–1575

Aoki K, Nakamura H, Suzuki H, Matsuo K, Kataoka K, Shimamura T, Motomura K, Ohka F, Shiina S, Yamamoto T, Nagata Y, Yoshizato T, Mizoguchi M, Abe T, Momii Y, Muragaki Y, Watanabe R, Ito I, Sanada M, Yajima H, Morita N, Takeuchi I, Miyano S, Wakabayashi T,

Ogawa S, Natsume A (2018) Prognostic relevance of genetic alterations in diffuse lower-grade gliomas. Neuro-Oncology 20:66–77

Appay R, Dehais C, Maurage CA, Alentorn A, Carpentier C, Colin C, Ducray F, Escande F, Idbaih A, Kamoun A, Marie Y, Mokhtari K, Tabouret E, Trabelsi N, Uro-Coste E, Delattre JY, Figarella-Branger D, POLA Network (2019) CDKN2A homozygous deletion is a strong adverse prognosis factor in diffuse malignant IDH-mutant gliomas. Neuro-Oncology 21: 1519–1528

Avninder S, Sharma MC, Deb P, Mehta VS, Karak AK, Mahapatra AK, Sarkar C (2006) Gemistocytic astrocytomas: histomorphology, proliferative potential and genetic alterations–a study of 32 cases. J Neuro-Oncol 78:123–127

Bai H, Bai S, Li X, Zhang Y, Li Y, He F, Cheng W (2021) Establishment and validation of the detection of TERT promoter mutations by human gliomas U251 cell lines. Biomed Res Int 2021:3271395

Bailey P, Cushing H (1926) A classification of the tumors of the glioma group on a histogenetic basis with a correlated study of prognosis. JB Lippincott, Philadelphia

Bale TA, Jordan JT, Rapalino O, Ramamurthy N, Jessop N, DeWitt JC, Nardi V, Alvarez MM, Frosch M, Batchelor TT, Louis DN, Iafrate AJ, Cahill DP, Lennerz JK (2019) Financially effective test algorithm to identify an aggressive, EGFR-amplified variant of IDH-wildtype, lower-grade diffuse glioma. Neuro-Oncology 21:596–605

Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A (2008) Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 116:597–602

Banerjee M, Dinda AK, Sinha S, Sarkar C, Mathur M (1996) C-myc oncogene expression and cell proliferation in mixed oligo-astrocytoma. Int J Cancer 65:730–733

Becker LE, Sharma MC, Rorke LB (2000) Medulloepithelioma. In: Kleihues P, Vavenee WK (eds) Pathology and genetics of tumours of the nervous system, 3rd edn. IARC, Lyon, pp 124–126

Becker AP, Scapulatempo-Neto C, Carloni AC, Paulino A, Sheren J, Aisner DL, Musselwhite E, Clara C, Machado HR, Oliveira RS, Neder L, Varella-Garcia M, Reis RM (2015) KIAA1549: BRAF gene fusion and FGFR1 hotspot mutations are prognostic factors in pilocytic astrocytomas. J Neuropathol Exp Neurol 74(7):743–754

Beiko J, Suki D, Hess KR, Fox BD, Cheung V, Cabral M, Shonka N, Gilbert MR, Sawaya R, Prabhu SS, Weinberg J, Lang FF, Aldape KD, Sulman EP, Rao G, McCutcheon IE, Cahill DP (2014) IDH1 mutant malignant astrocytomas are more amenable to surgical resection and have a survival benefit associated with maximal surgical resection. Neuro-Oncology 16:81–91

Bender S, Tang Y, Lindroth AM, Hovestadt V, Jones DTW, Kool M, Zapatka M, Northcott PA, Sturm D, Wang W, Radlwimmer B, Højfeldt JW, Truffaux N, Castel D, Schubert S, Ryzhova M, Şeker-Cin H, Gronych J, Johann PD, Stark S, Meyer J, Milde T, Schuhmann M, Ebinger M, Monoranu C-M, Ponnuswami A, Chen S, Jones C, Witt O, Collins VP, von Deimling A, Jabado N, Puget S, Grill J, Helin K, Korshunov A, Lichter P, Monje M, Plass C, Cho Y-J, Pfister SM (2013) Reduced H3K27me3 and DNA hypomethylation are major drivers of gene expression in K27M mutant pediatric high-grade gliomas. Cancer Cell 24(5):660–672. https://doi.org/10.1016/j.ccr.2013.10.006

Bettegowda C, Agrawal N, Jiao Y, Sausen M, Wood LD, Hruban RH, Rodriguez FJ, Cahill DP, McLendon R, Riggins G, Velculescu VE, Oba-Shinjo SM, Marie SK, Vogelstein B, Bigner D, Yan H, Papadopoulos N, Kinzler KW (2011) Mutations in CIC and FUBP1 contribute to human oligodendroglioma. Science 333:1453–1455

Brandner S, Kleinschmidt-DeMasters BK, Sarkar C (2021) CNS paraganglioma. In: WHO classification of tumours of the central nervous system, 5th edn. IARC, Lyon

Brat DJ, Aldape K, Colman H, Figrarella-Branger D, Fuller GN, Giannini C, Holland EC, Jenkins RB, Kleinschmidt-DeMasters B, Komori T, Kros JM, Louis DN, McLean C, Perry A, Reifenberger G, Sarkar C, Stupp R, van den Bent MJ, von Deimling A, Weller M (2020) cIMPACT-NOW update 5: recommended grading criteria and terminologies for IDH-mutant astrocytomas. Acta Neuropathol 139:603–608

Bui NQ, Przybyl J, Trabucco SE, Frampton G, Hastie T, van de Rijn M, Ganjoo KN (2019) A clinico-genomic analysis of soft tissue sarcoma patients reveals CDKN2A deletion as a biomarker for poor prognosis. Clin Sarcoma Res 9(1). https://doi.org/10.1186/s13569-019-0122-5

Butler M, Pongor L, Su YT, Xi L, Raffeld M, Quezado M, Trepel J, Aldape K, Pommier Y, Wu J (2020) MGMT status as a clinical biomarker in glioblastoma. Trends Cancer 6:380–391

Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, Hammond RR, Silver JS, Stark PC, Macdonald DR, Ino Y, Ramsay DA, Louis DN (1998) Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 90(19):1473–1479

Chatterjee D, Radotra BD, Kumar N, Vasishta RK, Gupta SK (2018) IDH1, ATRX, and BRAFV600E mutation in astrocytic tumors and their significance in patient outcome in north Indian population. Surg Neurol Int 9:29

Chattopadhyay P, Rathore A, Mathur M, Sarkar C, Mahapatra AK, Sinha S (1997) Loss of heterozygosity of a locus on 17p13.3, independent of p53, is associated with higher grades of astrocytic tumours. Oncogene 15:871–874

Chen X, Pan C, Zhang P, Xu C, Sun Y, Yu H, Wu Y, Geng Y, Zuo P, Wu Z, Zhang J, Zhang L (2017) BRAF V600E mutation is a significant prognosticator of the tumour regrowth rate in brainstem gangliogliomas. J Clin Neurosci 46:50–57

Cimino PJ, Zager M, McFerrin L, Wirsching HG, Bolouri H, Hentschel B, von Deimling A, Jones D, Reifenberger G, Weller M, Holland EC (2017) Multidimensional scaling of diffuse gliomas: application to the 2016 World Health Organization classification system with prognostically relevant molecular subtype discovery. Acta Neuropathol Commun 5:39

Cohen AL, Holmen SL, Colman H (2013) IDH1 and IDH2 mutations in gliomas. Curr Neurol Neurosci Rep 13:345

Coleman C, Stoller S, Grotzer M, Stucklin AG, Nazarian J, Mueller S (2020) Pediatric hemispheric high-grade glioma: targeting the future. Cancer Metastasis Rev 39:245–260

Cong YS, Wen J, Bacchetti S (1999) The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. Hum Mol Genet 8:137–142

Coons SW, Johnson PC, Scheithauer BW, Yates AJ, Pearl DK (1997) Improving diagnostic accuracy and interobserver concordance in the classification and grading of primary gliomas. Cancer 79:1381–1393

Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SM (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462:739–744

Das P, Puri T, Jha P, Pathak P, Joshi N, Suri V, Sharma MC, Sharma BS, Mahapatra AK, Suri A, Sarkar C (2011) A clinicopathological and molecular analysis of glioblastoma multiforme with long-term survival. J Clin Neurosci 18:66–70

Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA (2002) Mutations of the BRAF gene in human cancer. Nature 417:949–954

De Witt Hamer PC (2010) Small molecule kinase inhibitors in glioblastoma: a systematic review of clinical studies. Neuro-Oncology 12(3):304–316. https://doi.org/10.1093/neuonc/nop068

Deb P, Sharma MC, Mahapatra AK, Agarwal D, Sarkar C (2005) Glioblastoma multiforme with long term survival. Neurol India 53:329

Deb P, Sharma MC, Tripathi M, Sarat P, Gupta A, Sarkar C (2006) Expression of CD34 as a novel marker for glioneuronal lesions associated with chronic intractable epilepsy. Neuropathol Appl Neurobiol 32:461–468

Dinda AK, Sarkar C, Roy S (1990) Rosenthal fibres: an immunohistochemical, ultrastructural and immunoelectron microscopic study. Acta Neuropathol 79:456–460

Dinda AK, Sarkar C, Roy S, Kharbanda KU (1992) Immunohistochemical, ultrastructural & immunoelectron microscopic study of glial fibrillary acidic protein in corpora amylacea. Indian J Med Res 96:245–249

Dinda AK, Kharbanda K, Sarkar C, Roy S, Mathur M, Banerji AK (1993a) In-vivo proliferative potential of primary human brain tumors; its correlation with histological classification and morphological features: I gliomas. Pathology 25:4–9

Dinda AK, Sarkar C, Roy S, Kharbanda K, Mathur M, Khosla AK, Banerji AK (1993b) A transmission and scanning electron microscopic study of tumoral and peritumoral microblood vessels in human gliomas. J Neuro-Oncol 16:149–158

Dodgshun AJ, SantaCruz N, Hwang J, Ramkissoon SH, Malkin H, Bergthold G, Manley P, Chi S, MacGregor D, Goumnerova L, Sullivan M, Ligon K, Beroukhim R, Herrington B, Kieran MW, Hansford JR, Bandopadhayay P (2016) Disseminated glioneuronal tumors occurring in childhood: treatment outcomes and BRAF alterations including V600E mutation. J Neuro-Oncol 128:293–302

Eckel-Passow JE, Lachance DH, Molinaro AM, Walsh KM, Decker PA, Sicotte H, Pekmezci M, Rice T, Kosel ML, Smirnov IV, Sarkar G, Caron AA, Kollmeyer TM, Praska CE, Chada AR, Halder C, Hansen HM, McCoy LS, Bracci PM, Marshall R, Zheng S, Reis GF, Pico AR, O'Neill BP, Buckner JC, Giannini C, Huse JT, Perry A, Tihan T, Berger MS, Chang SM, Prados MD, Wiemels J, Wiencke JK, Wrensch MR, Jenkins RB (2015) Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. N Engl J Med 372:2499–2508

Ellison DW, Hawkins C, Jones DTW, Onar-Thomas A, Pfister SM, Reifenberger G, Louis DN (2019) cIMPACT-NOW update 4: diffuse gliomas characterized by MYB, MYBL1, or FGFR1 alterations or BRAFV600E mutation. Acta Neuropathol 137:683–687

Ellison DW, Clifford SC, Kaur K, Korshunov A, Northcott PA, Taylor MD (2021) Medulloblastoma, WNT-activated. Glioblastoma, IDH-wildtype. In: WHO classification of tumours of the central nervous system, 5th edn. IARC, Lyon

Fontana R, Ranieri M, La Mantia G, Vivo M (2019) Dual role of the alternative reading frame ARF protein in cancer. Biomol Ther 9:87

Fu Y, Zheng S, Zheng Y, Huang R, An N, Liang A, Hu C (2012) Glioma derived isocitrate dehydrogenase-2 mutations induced up-regulation of HIF-1α and β-catenin signaling: possible impact on glioma cell metastasis and chemo-resistance. Int J Biochem Cell Biol 44:770–775

Gierke M, Sperveslage J, Schwab D, Beschorner R, Ebinger M, Schuhmann MU, Schittenhelm J (2016) Analysis of IDH1-R132 mutation, BRAF V600 mutation and KIAA1549-BRAF fusion transcript status in central nervous system tumors supports pediatric tumor classification. J Cancer Res Clin Oncol 142:89–100

Gillet E, Alentorn A, Doukouré B, Mundwiller E, van Thuijl HF, Reijneveld JC, Medina JA, Liou A, Marie Y, Mokhtari K, Hoang-Xuan K, Sanson M, Delattre JY, Idbaih A (2014) TP53 and p53 statuses and their clinical impact in diffuse low grade gliomas. J Neuro-Oncol 118:131–139

Goodenberger ML, Jenkins RB (2012) Genetics of adult glioma. Cancer Genet 205:613–621

Guo Y, Long J, Lei S (2019) Promoter methylation as biomarkers for diagnosis of melanoma: a systematic review and meta-analysis. J Cell Physiol 234(5):7356–7367. https://doi.org/10.1002/jcp.27495

Haase S, Garcia-Fabiani MB, Carney S, Altshuler D, Núñez FJ, Méndez FM, Núñez F, Lowenstein PR, Castro MG (2018) Mutant ATRX: uncovering a new therapeutic target for glioma. Expert Opin Ther Targets 22:599–613

Haase S, Nuñez FM, Gauss JC, Thompson S, Brumley E, Lowenstein P, Castro MG (2020) Hemispherical pediatric high-grade glioma: molecular basis and therapeutic opportunities. Int J Mol Sci 21:9654

Hakar MH, Wood MD (2020) Updates in pediatric glioma pathology. Surg Pathol Clin 13:801–816

Haque F, Varlet P, Puntonet J, Storer L, Bountali A, Rahman R, Grill J, Carcaboso AM, Jones C, Layfield R, Grundy RG (2017) Evaluation of a novel antibody to define histone 3.3 G34R mutant brain tumours. Acta Neuropathol Commun 5:45

Hawkins C, Walker E, Mohamed N, Zhang C, Jacob K, Shirinian M, Alon N, Kahn D, Fried I, Scheinemann K, Tsangaris E, Dirks P, Tressler R, Bouffet E, Jabado N, Tabori U (2011) BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. Clin Cancer Res 17:4790–4798

Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997–1003

Heidenreich B, Rachakonda PS, Hosen I, Volz F, Hemminki K, Weyerbrock A, Kumar R (2015) TERT promoter mutations and telomere length in adult malignant gliomas and recurrences. Oncotarget 6:10617–10633

Horbinski C, Hamilton RL, Nikiforov Y, Pollack IF (2010) Association of molecular alterations, including BRAF, with biology and outcome in pilocytic astrocytomas. Acta Neuropathol 119: 641–649

Hotta T, Saito Y, Fujita H, Mikami T, Kurisu K, Kiya K, Uozumi T, Isowa G, Ishizaki K, Ikenaga M (1994) O6-alkylguanine-DNA alkyltransferase activity of human malignant glioma and its clinical implications. J Neuro-Oncol 21:135–140

Huang J, Yu J, Tu L, Huang N, Li H, Luo Y (2019) Isocitrate dehydrogenase mutations in glioma: from basic discovery to therapeutics development. Front Oncol 9:506

Ikemura M, Shibahara J, Mukasa A, Takayanagi S, Aihara K, Saito N, Aburatani H, Fukayama M (2016) Utility of ATRX immunohistochemistry in diagnosis of adult diffuse gliomas. Histopathology 69:260–267

Ino Y, Betensky RA, Zlatescu MC, Sasaki H, Macdonald DR, Stemmer-Rachamimov AO, Ramsay DA, Cairncross JG, Louis DN (2001) Molecular subtypes of anaplastic oligodendroglioma: implications for patient management at diagnosis. Clin Cancer Res 7:839–845

Jaeckle KA, Ballman KV, van den Bent M, Giannini C, Galanis E, Brown PD, Jenkins RB, Cairncross JG, Wick W, Weller M, Aldape KD, Dixon JG, Anderson SK, Cerhan JH, Wefel JS, Klein M, Grossman SA, Schiff D, Raizer JJ, Dhermain F, Nordstrom DG, Flynn PJ, Vogelbaum MA (2021) CODEL: phase III study of RT, RT + TMZ, or TMZ for newly diagnosed 1p/19q codeleted oligodendroglioma. Analysis from the initial study design. Neuro-Oncology 23:457–467

Jafri MA, Ansari SA, Alqahtani MH, Shay JW (2016) Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med 8:69

Jain A, Sharma MC, Suri V, Kale SS, Mahapatra AK, Tatke M, Chacko G, Pathak A, Santosh V, Nair P, Husain N, Sarkar C (2011) Spectrum of pediatric brain tumors in India: a multi-institutional study. Neurol India 59:208–211

Jha P, Agarwal S, Pathak P, Srivastava A, Suri V, Sharma MC, Chosdol K, Srivastava T, Gupta D, Gupta A, Suri A, Sarkar C (2010a) Heterozygosity status of 1p and 19q and its correlation with p53 protein expression and EGFR amplification in patients with astrocytic tumors: novel series from India. Cancer Genet Cytogenet 198:126–134

Jha P, Suri V, Jain A, Sharma MC, Pathak P, Jha P, Srivastava A, Suri A, Gupta D, Chosdol K, Chattopadhyay P, Sarkar C (2010b) O6-methylguanine DNA methyltransferase gene promoter methylation status in gliomas and its correlation with other molecular alterations: first Indian report with review of challenges for use in customized treatment. Neurosurgery 67:1681–1691

Jha P, Suri V, Sharma V, Singh G, Sharma MC, Pathak P, Chosdol K, Jha P, Suri A, Mahapatra AK, Kale SS, Sarkar C (2011a) IDH1 mutations in gliomas: first series from a tertiary care centre in India with comprehensive review of literature. Exp Mol Pathol 91:385–393

Jha P, Sarkar C, Pathak P, Sharma MC, Kale SS, Gupta D, Chosdol K, Suri V (2011b) Detection of allelic status of 1p and 19q by microsatellite-based PCR versus FISH: limitations and advantages in application to patient management. Diagn Mol Pathol 20:40–47

Jha P, Jha P, Pathak P, Chosdol K, Suri V, Sharma MC, Kumar G, Singh M, Mahapatra AK, Sarkar C (2011c) TP53 polymorphisms in gliomas from Indian patients: study of codon 72 genotype, rs1642785, rs1800370 and 16 base pair insertion in intron-3. Exp Mol Pathol 90:167–172

Jha P, Suri V, Singh G, Jha P, Purkait S, Pathak P, Sharma V, Sharma MC, Suri A, Gupta D, Mahapatra AK, Sarkar C (2011d) Characterization of molecular genetic alterations in GBMs highlights a distinctive molecular profile in young adults. Diagn Mol Pathol 20:225–232

Jha P, Agrawal R, Pathak P, Kumar A, Purkait S, Mallik S, Suri V, Chand Sharma M, Gupta D, Suri A, Sharma BS, Julka PK, Kulshreshtha R, Sarkar C (2015) Genome-wide small noncoding RNA profiling of pediatric high-grade gliomas reveals deregulation of several miRNAs, identifies downregulation of snoRNA cluster HBII-52 and delineates H3F3A and TP53 mutant-specific miRNAs and snoRNAs. Int J Cancer 137:2343–2353

Jha P, Manjunath N, Singh J, Mani K, Garg A, Kaur K, Sharma MC, Raheja A, Suri A, Sarkar C, Suri V (2019) Analysis of PD-L1 expression and T cell infiltration in different molecular subgroups of diffuse midline gliomas. Neuropathology 39:413–424

Jiao Y, Killela PJ, Reitman ZJ, Rasheed AB, Heaphy CM, de Wilde RF, Rodriguez FJ, Rosemberg S, Oba-Shinjo SM, Nagahashi Marie SK, Bettegowda C, Agrawal N, Lipp E, Pirozzi C, Lopez G, He Y, Friedman H, Friedman AH, Riggins GJ, Holdhoff M, Burger P, McLendon R, Bigner DD, Vogelstein B, Meeker AK, Kinzler KW, Papadopoulos N, Diaz LA, Yan H (2012) Frequent ATRX, CIC, FUBP1 and IDH1 mutations refine the classification of malignant gliomas. Oncotarget 3:709–722

Johanns TM, Fu Y, Kobayashi DK, Mei Y, Dunn IF, Mao DD, Kim AH, Dunn GP (2016) High incidence of TERT mutation in brain tumor cell lines. Brain Tumor Pathol 33:222–227

Jones DT, Kocialkowski S, Liu L, Pearson DM, Bäcklund LM, Ichimura K, Collins VP (2008) Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res 68:8673–8677

Kakkar A, Suri V, Jha P, Srivastava A, Sharma V, Pathak P, Sharma MC, Sharma MS, Kale SS, Chosdol K, Phalak M, Sarkar C (2011) Loss of heterozygosity on chromosome 10q in glioblastomas, and its association with other genetic alterations and survival in Indian patients. Neurol India 59:254–261

Kakkar A, Majumdar A, Kumar A, Tripathi M, Pathak P, Sharma MC, Suri V, Tandon V, Chandra SP, Sarkar C (2016a) Alterations in BRAF gene, and enhanced mTOR and MAPK signaling in dysembryoplastic neuroepithelial tumors (DNTs). Epilepsy Res 127:141–151

Kakkar A, Nambirajan A, Kaur K, Kumar A, Mallick S, Suri V, Sarkar C, Kale SS, Garg A, Sharma MC (2016b) ATRX loss in glioneuronal tumors with neuropil-like islands indicates similarity to diffuse astrocytic tumors. J Neuro-Oncol 130:63–68

Kakkar A, Majumdar A, Pathak P, Kumar A, Kumari K, Tripathi M, Sharma MC, Suri V, Tandon V, Chandra SP, Sarkar C (2017) BRAF gene alterations and enhanced mammalian target of rapamycin signaling in gangliogliomas. Neurol India 65:1076–1082

Karak AK, Singh R, Tandon PN, Sarkar C (2000) A comparative survival evaluation and assessment of interclassification concordance in adult supratentorial astrocytic tumors. Pathol Oncol Res 6:46–52

Karpel-Massler G, Nguyen TTT, Shang E, Siegelin MD (2019) Novel IDH1-targeted glioma therapies. CNS Drugs 33:1155–1166

Karremann M, Gielen GH, Hoffmann M, Wiese M, Colditz N, Warmuth-Metz M, Bison B, Claviez A, van Vuurden DG, von Bueren AO, Gessi M, Kühnle I, Hans VH, Benesch M, Sturm D, Kortmann RD, Waha A, Pietsch T, Kramm CM (2018) Diffuse high-grade gliomas with H3 K27M mutations carry a dismal prognosis independent of tumor location. Neuro-Oncology 20:123–131

Karsy M, Guan J, Cohen AL, Jensen RL, Colman H (2017) New molecular considerations for glioma: IDH, ATRX, BRAF, TERT, H3 K27M. Curr Neurol Neurosci Rep 17:19

Kernohan JW, Mabon RF (1949) A simplified classification of the gliomas. Proc Staff Meet Mayo Clin 24(3):71–75

Khanna G, Pathak P, Suri V, Sharma MC, Chaturvedi S, Ahuja A, Bhardwaj M, Garg A, Sarkar C, Sharma R (2018) Immunohistochemical and molecular genetic study on epitheliod glioblastomas: series of 7 cases with review of literature. Pathol Res Pract 22:1–8

Kharbanda K, Sarkar C, Dinda AK, Karak AK, Mathur M, Roy S (1993) Morphological appearance, growth kinetics and glial fibrillary acidic protein (GFAP) expression in primary in vitro explant culture of astrocytic neoplasms. Acta Oncol 32:301–306

Kharbanda K, Dinda AK, Sarkar C, Karak AK, Dhir R, Mathur M, Roy S (1995) A correlative study of in vivo and in vitro labeling index using bromodeoxyuridine in human brain tumors. J Neuro-Oncol 23:185–190

Kilday JP, Bartels UK, Bouffet E (2014) Targeted therapy in pediatric low-grade glioma. Curr Neurol Neurosci Rep 14:441

Kim HS, Kwon MJ, Song JH, Kim ES, Kim HY, Min KW (2018) Clinical implications of TERT promoter mutation on IDH mutation and MGMT promoter methylation in diffuse gliomas. Pathol Res Pract 214:881–888

Kloosterhof NK, Bralten LB, Dubbink HJ, French PJ, van den Bent MJ (2011) Isocitrate dehydrogenase-1 mutations: a fundamentally new understanding of diffuse glioma? Lancet Oncol 12:83–91

Korshunov A, Sycheva R, Gorelyshev S, Golanov A (2005) Clinical utility of fluorescence in situ hybridization (FISH) in nonbrainstem glioblastomas of childhood. Mod Pathol 18:1258–1263

Korshunov A, Sarkar C, McLendon R, Ng H-K, Judkins AR, Huang A, Pfister S, Kool M, Eberhart CG, Wesseling P, Fuller GN (2016) Embryonal tumor with multilayered rosettes, C19MC-altered. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO classification of tumours of the central nervous system, revised, 4th edn. IARC, Lyon, pp 201–205

Korshunov A, Casalini B, Chavez L, Hielscher T, Sill M, Ryzhova M, Sharma T, Schrimpf D, Stichel D, Capper D, Reuss DE, Sturm D, Absalyamova O, Golanov A, Lambo S, Bewerunge-Hudler M, Lichter P, Herold-Mende C, Wick W, Pfister SM, Kool M, Jones DTW, von Deimling A, Sahm F (2019) Integrated molecular characterization of IDH-mutant glioblastomas. Neuropathol Appl Neurobiol 45:108–118

Kumar A, Pathak P, Purkait S, Faruq M, Jha P, Mallick S, Suri V, Sharma MC, Suri A, Sarkar C (2015) Oncogenic KIAA1549-BRAF fusion with activation of the MAPK/ERK pathway in pediatric oligodendrogliomas. Cancer Genet 208:91–95

Kumar A, Nayak S, Pathak P, Purkait S, Malgulawar PB, Sharma MC, Suri V, Mukhopadhyay A, Suri A, Sarkar C (2018) Identification of miR-379/miR-656 (C14MC) cluster downregulation and associated epigenetic and transcription regulatory mechanism in oligodendrogliomas. J Neuro-Oncol 139:23–31

Kumari K, Sharma MC, Kakkar A, Malgulwar PB, Pathak P, Suri V, Sarkar C, Chandra SP, Faruq M, Gupta RK, Saran RK (2016) Role of mTOR signaling pathway in the pathogenesis of subependymal giant cell astrocytoma - a study of 28 cases. Neurol India 64:988–994

Labussière M, Boisselier B, Mokhtari K, Di Stefano AL, Rahimian A, Rossetto M, Ciccarino P, Saulnier O, Paterra R, Marie Y, Finocchiaro G, Sanson M (2014) Combined analysis of TERT, EGFR, and IDH status defines distinct prognostic glioblastoma classes. Neurology 83:1200–1206

Lassaletta A, Scheinemann K, Zelcer SM, Hukin J, Wilson BA, Jabado N, Carret AS, Lafay-Cousin L, Larouche V, Hawkins CE, Pond GR, Poskitt K, Keene D, Johnston DL, Eisenstat DD, Krishnatry R, Mistry M, Arnoldo A, Ramaswamy V, Huang A, Bartels U, Tabori U, Bouffet E (2016) Phase II weekly vinblastine for chemotherapy-naïve children with progressive low-grade glioma: a Canadian Pediatric Brain Tumor Consortium Study. J Clin Oncol 34:3537–3543

Li J, Li Z, Zhang C, Zhang C, Wang H (2020) Male patients with TERT mutation may be more likely to benefit from immunotherapy, especially for melanoma. Aging 12:17288–17294

Lin A, Rodriguez FJ, Karajannis MA, Williams SC, Legault G, Zagzag D, Burger PC, Allen JC, Eberhart CG, Bar EE (2012) BRAF alterations in primary glial and glioneuronal neoplasms of the central nervous system with identification of 2 novel KIAA1549:BRAF fusion variants. J Neuropathol Exp Neurol 71:66–72

Lopes MBS, Wiestler OD, Stemmer Rachamimov AO, Sharma MC (2007) Tuberous sclerosis complex and subependymal giant cell astrocytoma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO classification of tumours of the central nervous system, 4th edn. IARC, Lyon, pp 218–221

Lopes MBS, Wiestler OD, Stemmer Rachamimov AO, Sharma MC, Vinters HV, Santosh V (2016) Subependymal giant cell astrocytoma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO classification of tumours of the central nervous system, revised, 4th edn. IARC, Lyon, pp 90–92

Lopes MBS, Rodriguez FJ, Santosh V, Sharma MC, Stemmer-Rachamimov AO (2021) Tuberous sclerosis. In: WHO classification of tumours of the central nervous system, 5th edn. IARC, Lyon

Louis DN (1994) The p53 gene and protein in human brain tumors. J Neuropathol Exp Neurol 53: 11–21

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2016) WHO classification of tumours of the central nervous system, revised, 4th edn. IARC, Lyon

Louis DN, Wesseling P, Aldape K, Brat DJ, Capper D, Cree IA, Eberhart C, Figarella-Branger D, Fouladi M, Fuller GN, Giannini C, Haberler C, Hawkins C, Komori T, Kros JM, Ng HK, Orr BA, Park SH, Paulus W, Perry A, Pietsch T, Reifenberger G, Rosenblum M, Rous B, Sahm F, Sarkar C, Solomon DA, Tabori U, van den Bent MJ, von Deimling A, Weller M, White VA, Ellison DW (2020) cIMPACT-NOW update 6: new entity and diagnostic principle recommendations of the cIMPACT-Utrecht meeting on future CNS tumor classification and grading. Brain Pathol 30(4):844–856

Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R, von Deimling A, Ellison DW (2021a) The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol 23(8): 1231–1251

Louis DN, Aldape KD, Capper D, Giannini C, Horbinski CM, Ng HK, Perry A, Reifenberger G, Sarkar C, Soffietti R, Suvà ML, Wick W (2021b) Glioblastoma, IDH-wildtype. In: WHO classification of tumours of the central nervous system, 5th edn. IARC, Lyon

Low KC, Tergaonkar V (2013) Telomerase: central regulator of all of the hallmarks of cancer. Trends Biochem Sci 38:426–434

Malik A, Deb P, Sharma MC, Sarkar C (2006) Neuropathological spectrum of pilocytic astrocytoma-an Indian series of 120 cases. Pathol Oncol Res 12:164–171

Malmström A, Grønberg BH, Marosi C, Stupp R, Frappaz D, Schultz H, Abacioglu U, Tavelin B, Lhermitte B, Hegi ME, Rosell J, Henriksson R, Nordic Clinical Brain Tumour Study Group (NCBTSG) (2012) Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. Lancet Oncol 13:916–926

Manjunath N, Jha P, Singh J, Raheja A, Kaur K, Suri A, Garg A, Sharma MC, Sarkar C, Mohan M, Mani K, Suri V (2021) Clinico-pathological and molecular characterization of diffuse midline gliomas: is there a prognostic significance? Neurol Sci 42:925–934

Mansouri A, Hachem LD, Mansouri S, Nassiri F, Laperriere NJ, Xia D, Lindeman NI, Wen PY, Chakravarti A, Mehta MP, Hegi ME, Stupp R, Aldape KD, Zadeh G (2019) MGMT promoter methylation status testing to guide therapy for glioblastoma: refining the approach based on emerging evidence and current challenges. Neuro-Oncology 21:167–178

McLendon RE, Judkins AR, Eberhart CG, Fuller GN, Sarkar C, Ng HK (2007) Central nervous system primitive neuroectodermal tumors. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds) WHO classification of tumors of the central nervous system, 4th edn. IARC, Lyon, pp 141–146

McLendon RE, Ng HK, Judkins AR, Huang A, Eberhart CG, Kool M, Fuller GN, Pfister S, Sarkar C (2016) Other CNS embryonal tumours. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Ellison DW, Figarella-Branger D, Perry A, Reifenberger G, Von Deimling A (eds) WHO classification of tumours of the central nervous system, revised, 4th edn. IARC, Lyon, pp 206–208

Misra A, Chattopadhyay P, Dinda AK, Sarkar C, Mahapatra AK, Hasnain SE, Sinha S (2000) Extensive intra-tumor heterogeneity in primary human glial tumors as a result of locus non-specific genomic alterations. J Neuro-Oncol 48:1–2

Mizoguchi M, Yoshimoto K, Ma X, Guan Y, Hata N, Amano T, Nakamizo A, Suzuki SO, Iwaki T, Sasaki T (2012) Molecular characteristics of glioblastoma with 1p/19q co-deletion. Brain Tumor Pathol 29:148–153

Mosrati MA, Malmström A, Lysiak M, Krysztofiak A, Hallbeck M, Milos P, Hallbeck AL, Bratthäll C, Strandéus M, Stenmark-Askmalm M, Söderkvist P (2015) TERT promoter mutations and polymorphisms as prognostic factors in primary glioblastoma. Oncotarget 6: 16663–16673

Nayak A, Ralte AM, Sharma MC, Singh VP, Mahapatra AK, Mehta VS, Sarkar C (2004) p53 protein alterations in adult astrocytic tumors and oligodendrogliomas. Neurol India 52:228–232

Nayak S, Aich M, Kumar A, Sengupta S, Bajad P, Dhapola P, Paul D, Narta K, Purkait S, Mehani B, Suri A, Chakraborty D, Mukhopadhyay A, Sarkar C (2018) Novel internal regulators and candidate miRNAs within miR-379/miR-656 miRNA cluster can alter cellular phenotype of human glioblastoma. Sci Rep 8:7673

Ohgaki H, Kleihues P (2005) Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol 64:479–489

Olafson LR, Gunawardena M, Nixdorf S, McDonald KL, Rapkins RW (2020) The role of TP53 gain-of-function mutation in multifocal glioblastoma. J Neuro-Oncol 147:37–47

Ostrom QT, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C, Barnholtz-Sloan JS (2019) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012–2016. Neuro-Oncology 21(Suppl 5):v1–v100

Pal J, Patil V, Kumar A, Kaur K, Sarkar C, Somasundaram K (2018) Loss-of-function mutations in calcitonin receptor (CALCR) identify highly aggressive glioblastoma with poor outcome. Clin Cancer Res 24:1448–1458

Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812

Patel R, Leung HY (2012) Targeting the EGFR-family for therapy: biological challenges and clinical perspective. Curr Pharm Des 18:2672–2679

Patel B, Taiwo R, Kim AH, Dunn GP (2020) TERT, a promoter of CNS malignancies. Neurooncol Adv 2:vdaa025

Pathak P, Jha P, Purkait S, Sharma V, Suri V, Sharma MC, Faruq M, Suri A, Sarkar C (2015) Altered global histone-trimethylation code and H3F3A-ATRX mutation in pediatric GBM. J Neuro-Oncol 121:489–497

Pathak P, Kumar A, Jha P, Purkait S, Faruq M, Suri A, Suri V, Sharma MC, Sarkar C (2017) Genetic alterations related to BRAF-FGFR genes and dysregulated MAPK/ERK/mTOR signaling in adult pilocytic astrocytoma. Brain Pathol 27:580–589

Pekmezci M, Rice T, Molinaro AM, Walsh KM, Decker PA, Hansen H, Sicotte H, Kollmeyer TM, McCoy LS, Sarkar G, Perry A, Giannini C, Tihan T, Berger MS, Wiemels JL, Bracci PM, Eckel-Passow JE, Lachance DH, Clarke J, Taylor JW, Luks T, Wiencke JK, Jenkins RB, Wrensch MR (2017) Adult infiltrating gliomas with WHO 2016 integrated diagnosis: additional prognostic roles of ATRX and TERT. Acta Neuropathol 133:1001–1016

Pietrak B, Zhao H, Qi H, Quinn C, Gao E, Boyer JG, Concha N, Brown K, Duraiswami C, Wooster R, Sweitzer S, Schwartz B (2011) A tale of two subunits: how the neomorphic R132H IDH1 mutation enhances production of αHG. Biochemistry 50:4804–4812

Preusser M (2009) MGMT analysis at DNA, RNA and protein levels in glioblastoma tissue. Histol Histopathol 24:511–518

Purkait S, Jha P, Sharma MC, Suri V, Sharma M, Kale SS, Sarkar C (2013) CDKN2A deletion in pediatric versus adult glioblastomas and predictive value of p16 immunohistochemistry. Neuropathology 33:405–412

Purkait S, Sharma V, Jha P, Sharma MC, Suri V, Suri A, Sharma BS, Sarkar C (2015) EZH2 expression in gliomas: correlation with CDKN2A gene deletion/p16 loss and MIB-1 proliferation index. Neuropathology 35:421–431

Purkait S, Mallick S, Sharma V, Kumar A, Pathak P, Jha P, Biswas A, Julka PK, Gupta D, Suri A, Datt Upadhyay A, Suri V, Sharma MC, Sarkar C (2016a) Prognostic stratification of GBMs using combinatorial assessment of IDH1 mutation, MGMT promoter methylation, and TERT mutation status: experience from a tertiary care center in India. Transl Oncol 9:371–376

Purkait S, Sharma V, Kumar A, Pathak P, Mallick S, Jha P, Sharma MC, Suri V, Julka PK, Suri A, Sharma BS, Sarkar C (2016b) Expression of DNA methyltransferases 1 and 3B correlates with EZH2 and this 3-marker epigenetic signature predicts outcome in glioblastomas. Exp Mol Pathol 100:312–320

Purkait S, Mallick S, Sharma V, Kumar A, Pathak P, Jha P, Biswas A, Julka PK, Gupta D, Suri A, Upadhyay AD, Suri V, Sharma MC, Sarkar C (2016c) A simplified approach for molecular classification of glioblastomas (GBMs): experience from a tertiary care center in India. Brain Tumor Pathol 33:183–190

Purkait S, Miller CA, Kumar A, Sharma V, Pathak P, Jha P, Sharma MC, Suri V, Suri A, Sharma BS, Fulton RS, Kale SS, Dahiya S, Sarkar C (2017) ATRX in diffuse gliomas with its mosaic/heterogeneous expression in a subset. Brain Pathol 27:138–145

Ralte AM, Sharma MC, Karak AK, Mehta VS, Sarkar C (2001) Clinicopathological features, MIB-1 labeling index and apoptotic index in recurrent astrocytic tumors. Pathol Oncol Res 7:267–278

Rathore A, Kamarajan P, Mathur M, Sinha S, Sarkar C (1999) Simultaneous alterations of retinoblastoma and p53 protein expression in astrocytic tumors. Pathol Oncol Res 5:21–27

Reifenberger J, Reifenberger G, Liu L, James CD, Wechsler W, Collins VP (1994) Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. Am J Pathol 145:1175–1190

Reifenberger G, Wirsching HG, Knobbe-Thomsen CB, Weller M (2017) Advances in the molecular genetics of gliomas - implications for classification and therapy. Nat Rev Clin Oncol 14:434–452

Ringertz N (1950) Grading of gliomas. Acta Pathol Microbiol Scand 27:51–64

Roy S, Sarkar C, Tandon PN (1988) Use of markers in the study of brain tumors. Neurol India 36:351–356

Ryall S, Arnoldo A, Krishnatry R, Mistry M, Khor K, Sheth J, Ling C, Leung S, Zapotocky M, Guerreiro Stucklin A, Lassaletta A, Shago M, Tabori U, Hawkins CE (2017) Multiplex detection of pediatric low-grade glioma signature fusion transcripts and duplications using the NanoString nCounter System. J Neuropathol Exp Neurol 76:562–570

Ryall S, Zapotocky M, Fukuoka K, Nobre L, Stucklin AG, Julie B, Siddaway R, Li C, Pajovic S, Arnoldo A, Kowalski PE, Johnson M, Sheth J, Lassaletta A, Tatevossian RG, Orisme W, Qaddoumi I, Surrey LF, Li MM, Waanders AJ, Gilheeney S, Rosenblum M, Bale T, Tsang DS, Laperriere N, Kulkarni A, Ibrahim GM, Drake J, Dirks P, Taylor MD, Rutka JT, Laughlin S, Shroff M, Shago M, Hazrati L-N, D'Arcy C, Ramaswamy V, Bartels U, Huang A, Bouffet E, Karajannis MA, Santi M, Ellison DW, Tabori U, Cynthia H (2020) Integrated molecular and clinical analysis of 1000 pediatric low-grade gliomas. Cancer Cell 37(4):569–583.e5. https://doi.org/10.1016/j.ccell.2020.03.011

Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, Gilbert MR, Herndon JE II, McLendon RE, Mitchell DA, Reardon DA, Sawaya R, Schmittling RJ, Shi W, Vredenburgh JJ, Bigner DD (2010) Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J Clin Oncol 28:4722–4729

Santosh V (2012) Molecular pathology of diffuse low grade gliomas-impact on prognosis and therapy. In: Banerji D, Pauranik A (eds) Progress in clinical neurosciences, vol 2. Neurological Society of India, New Delhi, pp 115–123

Santosh V, Sravya P, Gupta T, Muzumdar D, Chacko G, Suri V, Epari S, Balasubramaniam A, Radotra BD, Chatterjee S, Sarkar C, Jalali R (2019) ISNO consensus guidelines for practical adaptation of the WHO 2016 classification of adult diffuse gliomas. Neurol India 67:173–182

Sarkar C, Roy S, Tandon PN (1988) Oligodendroglial tumors. An immunohistochemical and electron microscopic study. Cancer 61:1862–1866

Sarkar C, Sharma MC, Sudha K, Gaikwad S, Varma A (1997) A clinico-pathological study of 29 cases of gliosarcoma with special reference to two unique variants. Indian J Med Res 106: 229–235

Sarkar C, Rathore A, Chattopadhyaya P, Mahapatra AK, Sinha S (2000) Role of 17p13.3 chromosomal region in determining p53 protein immunopositivity in human astrocytic tumors. Pathology 32:84–88

Sarkar C, Ralte AM, Sharma MC, Mehta VS (2002) Recurrent astrocytic tumours-a study of p53 immunoreactivity and malignant progression. Br J Neurosurg 16:335–342

Sarkar C, Chattopadhyay P, Ralte AM, Mahapatra AK, Sinha S (2003) Loss of heterozygosity of a locus in the chromosomal region 17p13. 3 is associated with increased cell proliferation in astrocytic tumors. Cancer Genet Cytogenet 144:156–164

Sarkar C, Sinha S, Sharma MC, Kumar R, Mehta VS (2004) Supratentorial glioblastoma in adults: identification of subsets and their clinical correlation. Brain Tumor Pathol 21:7–12

Sarkar C, Karak AK, Nath N, Sharma MC, Mahapatra AK, Chattopadhyay P, Sinha S (2005) Apoptosis and proliferation: correlation with p53 in astrocytic tumours. J Neuro-Oncol 73:93–100

Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG (2018) Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): an abridged explanation and elaboration. J Natl Cancer Inst 110:803–811

Schiffman JD, Hodgson JG, VandenBerg SR, Flaherty P, Polley MY, Yu M, Fisher PG, Rowitch DH, Ford JM, Berger MS, Ji H, Gutmann DH, James CD (2010) Oncogenic BRAF mutation with CDKN2A inactivation is characteristic of a subset of pediatric malignant astrocytomas. Cancer Res 70:512–519

Schindler G, Capper D, Meyer J, Janzarik W, Omran H, Herold-Mende C, Schmieder K, Wesseling P, Mawrin C, Hasselblatt M, Louis DN, Korshunov A, Pfister S, Hartmann C, Paulus W, Reifenberger G, von Deimling A (2011) Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. Acta Neuropathol 121:397–405

Schumacher T, Bunse L, Pusch S, Sahm F, Wiestler B, Quandt J, Menn O, Osswald M, Oezen I, Ott M, Keil M, Balß J, Rauschenbach K, Grabowska AK, Vogler I, Diekmann J, Trautwein N, Eichmüller SB, Okun J, Stevanović S, Riemer AB, Sahin U, Friese MA, Beckhove P, von Deimling A, Wick W, Platten M (2014) A vaccine targeting mutant IDH1 induces antitumour immunity. Nature 512:324–327

Schwartzentruber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang DA, Tönjes M, Hovestadt V, Albrecht S, Kool M, Nantel A, Konermann C, Lindroth A, Jäger N, Rausch T, Ryzhova M, Korbel JO, Hielscher T, Hauser P, Garami M, Klekner A, Bognar L, Ebinger M, Schuhmann MU, Scheurlen W, Pekrun A, Frühwald MC, Roggendorf W, Kramm C, Dürken M, Atkinson J, Lepage P, Montpetit A, Zakrzewska M, Zakrzewski K, Liberski PP, Dong Z, Siegel P, Kulozik AE, Zapatka M, Guha A, Malkin D, Felsberg J, Reifenberger G, von Deimling A, Ichimura K, Collins VP, Witt H, Milde T, Witt O, Zhang C, Castelo-Branco P, Lichter P, Faury D, Tabori U, Plass C, Majewski J, Pfister SM, Jabado N (2012) Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature 482:226–231

Serrano M, Hannon GJ, Beach D (1993) A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366:704–707

Sharma S, Karak AK, Sarkar C, Gomathy G, Banerji AK, Schmitt HP (1996) A grading study of gliomas using computer aided malignancy classification and histologic morphometry. J Neuro-Oncol 27:75–85

Sharma MC, Ralte AM, Gaekwad S, Santosh V, Shankar SK, Sarkar C (2004) Subependymal giant cell astrocytoma—a clinicopathological study of 23 cases with special emphasis on histogenesis. Pathol Oncol Res 10:219–224

Sharma S, Sharma MC, Gupta DK, Sarkar C (2006) Angiogenic patterns and their quantitation in high grade astrocytic tumors. J Neuro-Oncol 79:19–30

Sharma V, Purkait S, Takkar S, Malgulwar PB, Kumar A, Pathak P, Suri V, Sharma MC, Suri A, Kale SS, Kulshreshtha R, Sarkar C (2016) Analysis of EZH2: micro-RNA network in low and high grade astrocytic tumors. Brain Tumor Pathol 33:117–128

Sharma V, Malgulwar PB, Purkait S, Patil V, Pathak P, Agrawal R, Kulshreshtha R, Mallick S, Julka PK, Suri A, Sharma BS, Suri V, Sharma MC, Sarkar C (2017) Genome-wide ChIP-seq analysis of EZH2-mediated H3K27me3 target gene profile highlights differences between low- and high-grade astrocytic tumors. Carcinogenesis 38:152–161

Shay JW, Bacchetti S (1997) A survey of telomerase activity in human cancer. Eur J Cancer 33: 787–791

Sherr CJ, Roberts JM (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev 13(12):1501–1512. https://doi.org/10.1101/gad.13.12.1501

Shukla B, Agarwal S, Suri V, Pathak P, Sharma MC, Gupta D, Sharma BS, Suri A, Halder A, Sarkar C (2009) Assessment of 1p/19q status by fluorescence in situ hybridization assay: a comparative study in oligodendroglial, mixed oligoastrocytic and astrocytic tumors. Neurol India 57:559–566

Smith JS, Perry A, Borell TJ, Lee HK, O'Fallon J, Hosek SM, Kimmel D, Yates A, Burger PC, Scheithauer BW, Jenkins RB (2000) Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J Clin Oncol 18:636–645

Srivastava T, Chattopadhyay P, Mahapatra AK, Sarkar C, Sinha S (2004) Increased hMSH2 protein expression in glioblastoma multiforme. J Neuro-Oncol 66:51–57

Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO, European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996

Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, Pfaff E, Tönjes M, Sill M, Bender S, Kool M, Zapatka M, Becker N, Zucknick M, Hielscher T, Liu XY, Fontebasso AM, Ryzhova M, Albrecht S, Jacob K, Wolter M, Ebinger M, Schuhmann MU, van Meter T, Frühwald MC, Hauch H, Pekrun A, Radlwimmer B, Niehues T, von Komorowski G, Dürken M, Kulozik AE, Madden J, Donson A, Foreman NK, Drissi R, Fouladi M, Scheurlen W, von Deimling A, Monoranu C, Roggendorf W, Herold-Mende C, Unterberg A, Kramm CM, Felsberg J, Hartmann C, Wiestler B, Wick W, Milde T, Witt O, Lindroth AM, Schwartzentruber J, Faury D, Fleming A, Zakrzewska M, Liberski PP, Zakrzewski K, Hauser P, Garami M, Klekner A, Bognar L, Morrissy S, Cavalli F, Taylor MD, van Sluis P, Koster J, Versteeg R, Volckmann R, Mikkelsen T, Aldape K, Reifenberger G, Collins VP, Majewski J, Korshunov A, Lichter P, Plass C, Jabado N, Pfister SM (2012) Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell 22:425–437

Sun ZL, Chan AK, Chen LC, Tang C, Zhang ZY, Ding XJ, Wang Y, Sun CR, Ng HK, Yao Y, Zhou LF (2015) TERT promoter mutated WHO grades II and III gliomas are located preferentially in the frontal lobe and avoid the midline. Int J Clin Exp Pathol 8:11485–11494

Sun Y, Bailey CP, Sadighi Z, Zaky W, Chandra J (2020) Pediatric high-grade glioma: aberrant epigenetics and kinase signaling define emerging therapeutic opportunities. J Neuro-Oncol 150 (1):17–26. https://doi.org/10.1007/s11060-020-03546-0

Suri V, Das P, Pathak P, Jain A, Sharma MC, Borkar SA, Suri A, Gupta D, Sarkar C (2009) Pediatric glioblastomas: a histopathological and molecular genetic study. Neuro-Oncology 11: 274–280

Suri V, Jha P, Sharma MC, Sarkar C (2011a) O6-methylguanine DNA methyltransferase gene promoter methylation in high-grade gliomas: a review of current status. Neurol India 59:229–235

Suri V, Jha P, Agarwal S, Pathak P, Sharma MC, Sharma V, Shukla S, Somasundaram K, Mahapatra AK, Kale SS, Sarkar C (2011b) Molecular profile of oligodendrogliomas in young patients. Neuro-Oncology 13:1099–1106

Taha H, Yehia M, Mahmoud M, El-Beltagy M, Ghabriel M, El-Naggar S (2015) Incidence of kiaa1549-braf fusion gene in Egyptian pediatric low grade glioma. Clin Transl Med 4:10

Takami H, Yoshida A, Fukushima S, Arita H, Matsushita Y, Nakamura T, Ohno M, Miyakita Y, Shibui S, Narita Y, Ichimura K (2015) Revisiting TP53 mutations and immunohistochemistry—a comparative study in 157 diffuse gliomas. Brain Pathol 25:256–265

Tateishi K, Yamamoto T (2019) IDH-mutant gliomas. In: Brain and spinal tumors-primary and secondary. IntechOpen, London

van den Bent MJ, Brandes AA, Taphoorn MJ, Kros JM, Kouwenhoven MC, Delattre JY, Bernsen HJ, Frenay M, Tijssen CC, Grisold W, Sipos L, Enting RH, French PJ, Dinjens WN, Vecht CJ, Allgeier A, Lacombe D, Gorlia T, Hoang-Xuan K (2013) Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. J Clin Oncol 31:344–350

van den Bent MJ, Baumert B, Erridge SC, Vogelbaum MA, Nowak AK, Sanson M, Brandes AA, Clement PM, Baurain JF, Mason WP, Wheeler H, Chinot OL, Gill S, Griffin M, Brachman DG, Taal W, Rudà R, Weller M, McBain C, Reijneveld J, Enting RH, Weber DC, Lesimple T, Clenton S, Gijtenbeek A, Pascoe S, Herrlinger U, Hau P, Dhermain F, van Heuvel I, Stupp R, Aldape K, Jenkins RB, Dubbink HJ, Dinjens WNM, Wesseling P, Nuyens S, Golfinopoulos V, Gorlia T, Wick W, Kros JM (2017) Interim results from the CATNON trial (EORTC study 26053-22054) of treatment with concurrent and adjuvant temozolomide for 1p/19q non-co-deleted anaplastic glioma: a phase 3, randomised, open-label intergroup study. Lancet 390: 1645–1653

Venneti S, Huse JT (2015) The evolving molecular genetics of low-grade glioma. Adv Anat Pathol 22:94–101

Vuorinen V, Hinkka S, Färkkilä M, Jääskeläinen J (2003) Debulking or biopsy of malignant glioma in elderly people - a randomised study. Acta Neurochir 145:5–10

Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W, Hegi ME (2010) MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat Rev Neurol 6:39–51

Weller M, van den Bent M, Tonn JC, Stupp R, Preusser M, Cohen-Jonathan-Moyal E, Henriksson R, Le Rhun E, Balana C, Chinot O, Bendszus M, Reijneveld JC, Dhermain F, French P, Marosi C, Watts C, Oberg I, Pilkington G, Baumert BG, Taphoorn MJB, Hegi M, Westphal M, Reifenberger G, Soffietti R, Wick W, European Association for Neuro-Oncology (EANO) Task Force on Gliomas (2017) European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. Lancet Oncol 18:e315–e329

Wen PY, Kesari S (2008) Malignant gliomas in adults. N Engl J Med 359(5):492–507

Wick W, Platten M, Meisner C, Felsberg J, Tabatabai G, Simon M, Nikkhah G, Papsdorf K, Steinbach JP, Sabel M, Combs SE, Vesper J, Braun C, Meixensberger J, Ketter R, Mayer-Steinacker R, Reifenberger G, Weller M, NOA-08 Study Group of Neuro-oncology Working Group (NOA) of German Cancer Society (2012) Temozolomide chemotherapy alone versus

radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. Lancet Oncol 13:707–715

Wood MD, Halfpenny AM, Moore SR (2019) Applications of molecular neuro-oncology - a review of diffuse glioma integrated diagnosis and emerging molecular entities. Diagn Pathol 14:29

Worst TS, Weis C-A, Stöhr R, Bertz S, Eckstein M, Otto W, Breyer J, Hartmann A, Bolenz C, Wirtz RM, Erben P (2018) CDKN2A as transcriptomic marker for muscle-invasive bladder cancer risk stratification and therapy decision-making. Sci Rep 8(1). https://doi.org/10.1038/s41598-018-32569-x

Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfort J, Qu C, Ding L, Huether R, Parker M, Zhang J, Gajjar A, Dyer MA, Mullighan CG, Gilbertson RJ, Mardis ER, Wilson RK, Downing JR, Ellison DW, Zhang J, Baker SJ, St. Jude Children's Research Hospital–-Washington University Pediatric Cancer Genome Project (2012) Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat Genet 44:251–253

Xia L, Zhang W, Gao L (2019) Clinical and prognostic effects of CDKN2A, CDKN2B and CDH13 promoter methylation on ovarian cancer: a study using meta-analysis and TCGA data. Biomarkers 24(6):1–29. https://doi.org/10.1080/1354750X.2019.1652685

Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765–773

Yen KE, Bittinger MA, Su SM, Fantin VR (2010) Cancer-associated IDH mutations: biomarker and therapeutic opportunities. Oncogene 29:6409–6417

Yip S, Butterfield YS, Morozova O, Chittaranjan S, Blough MD, An J, Birol I, Chesnelong C, Chiu R, Chuah E, Corbett R, Docking R, Firme M, Hirst M, Jackman S, Karsan A, Li H, Louis DN, Maslova A, Moore R, Moradian A, Mungall KL, Perizzolo M, Qian J, Roldan G, Smith EE, Tamura-Wells J, Thiessen N, Varhol R, Weiss S, Wu W, Young S, Zhao Y, Mungall AJ, Jones SJ, Morin GB, Chan JA, Cairncross JG, Marra MA (2012) Concurrent CIC mutations, IDH mutations, and 1p/19q loss distinguish oligodendrogliomas from other cancers. J Pathol 226:7–16

You H, Wu Y, Chang K, Shi X, Chen XD, Yan W, Li R (2017) Paradoxical prognostic impact of TERT promoter mutations in gliomas depends on different histological and genetic backgrounds. CNS Neurosci Ther 23:790–797

Yuan Y, Qi C, Maling G, Xiang W, Yanhui L, Ruofei L, Yunhe M, Jiewen L, Qing M (2016) TERT mutation in glioma: frequency, prognosis and risk. J Clin Neurosci 26:57–62

Zhang J, Wu G, Miller CP, Tatevossian RG, Dalton JD, Tang B, Orisme W, Punchihewa C, Parker M, Qaddoumi I, Boop FA, Lu C, Kandoth C, Ding L, Lee R, Huether R, Chen X, Hedlund E, Nagahawatte P, Rusch M, Boggs K, Cheng J, Becksfort J, Ma J, Song G, Li Y, Wei L, Wang J, Shurtleff S, Easton J, Zhao D, Fulton RS, Fulton LL, Dooling DJ, Vadodaria B, Mulder HL, Tang C, Ochoa K, Mullighan CG, Gajjar A, Kriwacki R, Sheer D, Gilbertson RJ, Mardis ER, Wilson RK, Downing JR, Baker SJ, Ellison DW, St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project (2013) Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat Genet 45:602–612

Zhang Y, Dube C, Gibert M Jr, Cruickshanks N, Wang B, Coughlan M, Yang Y, Setiady I, Deveau C, Saoud K, Grello C, Oxford M, Yuan F, Abounader R (2018) The p53 pathway in glioblastoma. Cancers (Basel) 10:297

Astroglial Iron Homeostasis and Neurodegenerative Diseases

Abhishek Mukherjee, Som Dev, Esha Ghosh, Somya Asthana, and Chinmay K. Mukhopadhyay

Abstract

Iron is essential for normal brain functioning for satisfying high demand of energy requirement, myelin synthesis, and neurotransmitter synthesis and for maintaining numerous other homeostatic functions. In contrary, iron deposition is detected in most of the neurodegenerative diseases including Parkinson's, Alzheimer's, as well as aging. Among glial cells, astroglia play pivotal role in supplying to and removing iron from neurons. Since iron is redox active in nature and potentially toxic particularly in conjunction with reactive oxygen species (ROS), maintaining astroglial iron homeostasis is crucial to avoid iron-induced neurodegeneration. In the last couple of decades, the understanding of the role of iron in biology has expanded substantially along with the discovery of many new players of iron homeostasis. The current chapter will focus on providing an update on astroglial iron homeostasis and its role in pathophysiology of neurodegenerative diseases.

Keywords

Astroglia · Iron · Neurodegeneration · Homeostasis · Oxidative stress

1 Introduction

Astrocyte, the most abundant glial cell, is considered as the key player in regulating brain homeostasis. Several lines of evidence suggest that it plays a pivotal role in maintaining the neuronal plasticity during development and disease. This very

A. Mukherjee · S. Dev · E. Ghosh · S. Asthana · C. K. Mukhopadhyay (✉)
Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, India
e-mail: ckm2300@jnu.ac.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_14

association of astrocytes probably makes them integrally related to the onset and progression of neurodegeneration. The change in astrocyte activity as a consequence of neuronal damage is termed as "reactive gliosis" that determines the fate of neurons. Astrocytes, under abnormal condition, secrete inflammatory mediators that can be contextually neuroprotective or neurotoxic. Also, proinflammatory response results in astrocyte activation that may lead astrocytes to acquire neuroprotective or detrimental phenotype. Recent evidences show that astrocyte activation primarily depends on oxidative stress that is highly correlated to the cytosolic iron levels. The redox-active free iron is highly toxic as it promotes detrimental reactive oxygen species (ROS) (Dev et al. 2015). Cells counterbalance such situations by storing free iron within cytosolic ferritin and quenching the generated ROS by endogenous antioxidant defense systems. The brain, due to its high metabolic activity, consumes large amount of oxygen, thus leading to an increased possibility of ROS formation (Mukhopadhyay et al. 2011; Singh et al. 2014). Neurons require large amount of iron for their normal functions. Thus, any disturbance in iron homeostasis may affect the viability of neurons by generating excessive ROS that cannot be counterbalanced by the endogenous antioxidant defense system. Recent therapeutic approaches to counter this include exogenous antioxidant therapy and targeting components of iron homeostasis. However, far less attention has been paid to astrocytes which under diseased condition may play instrumental roles in storing and/or releasing iron in order to protect neurons from iron-induced oxidative injury. Therefore, this chapter will primarily focus on exploring the role of astrocytes in maintaining their iron homeostasis as a protective measure for neuronal survival during neurodegeneration.

2 Role of Astrocytes in Maintenance of Neurons

Initially regarded as the housekeeping cells for maintaining neuronal microenvironment and function, astrocytes are now considered to be important counterparts of neurons for the activity of the central nervous system (CNS). They are found as the main suppliers of metabolites to neurons (Belanger et al. 2011). Catabolism and synthesis of new amino acids occur primarily in astrocytes for supplying molecular intermediates of various reaction cascades in the brain (Maragakis and Rothstein 2006). Pyruvate required for neuronal energy metabolism is supplied in the form of lactate derived from glucose metabolism within astrocytes (Danbolt 2001). Astrocytes also play important role in regulating neuronal pH and ion balance. Carbonic anhydrase in astrocytes converts neuron-generated carbon dioxide to bicarbonate (Svichar and Chesler 2003). Neurons act as transient oscillators whose synchronization leads to membrane resonance helping in amplification of inputs to define the functionality of neuronal network (Buskila et al. 2013, 2019; Laudanski et al. 2014). Changes in oscillation of individual neurons may change or disturb the resonance frequency to affect brain synchronicity (Ding et al. 2016). Extracellular ions like Na^+ and K^+ can affect the neuronal membrane properties to modulate the excitability of neurons. Astrocytes maintain the K^+ homeostasis in the brain by either

taking up excess K^+ from extracellular matrix or mediating K^+ spatial buffering, to prevent hyperexcitation of neurons (Verkhratsky and Nedergaard 2018; Buskila et al. 2019). Neuronal activity generates high amount of potassium which is taken up by potassium channels present in astrocytes. The expanded end feet of astrocytes act as a regulator of metabolite transportation between the blood and neurons (Dringen et al. 2007), simultaneously protecting other brain cells from toxic metal ions. Glutamate is the key excitatory neurotransmitter in CNS, and its regulation takes place via an intricate interplay of neurons and astrocytes. Glutamate transporters are localized on astrocyte membrane (Tanaka et al. 1997). The extracellular glutamate is taken up and converted to glutamine by astrocytes, which then replenishes the glutamine pool of presynaptic terminals for glutamate synthesis to help neurotransmission (Danbolt 2001). Astrocytes have the unique property to sense the released neurotransmitters at nearby synaptic sites, as they possess functional receptors on plasma membrane (Brambilla et al. 2014). This allows them to play important roles in the propagation of neuronal glutamate signaling by ATP-dependent calcium feed forward mechanism (Simard and Nedergaard 2004). Changes in Ca^+ oscillation in astrocytes have been found to alter the neuronal circuitry and behavioral outcome (Ma et al. 2016). Astrocytes have been found to control the functional segregation in different regions of the CNS, which has been further supported by their varied spatial and functional organizations (Takata and Hirase 2008; Matias et al. 2019). The close proximity of astrocytes to neurons in regulating synaptic transmission has led to the concept of tripartite synapse, an association of the pre- and postsynaptic neurons with the peri-synaptic astrocytes for the flow of information within the CNS (Perea et al. 2009).

3 Iron Is an Essential Component for Neuronal Function

Iron is considered as an important regulator for the maintenance of neuronal functions. It acts as a cofactor for several enzymes including tryptophan hydroxylase and tyrosine hydroxylase, which are responsible for the synthesis of neurotransmitters like serotonin, dopamine, and norepinephrine (Snyder 2011). Iron acts as the prosthetic group for enzymes that catalyzes the synthesis of GABA and L-glutamate and helps in dopamine catabolism. Iron acts as the cofactor for the rate limiting enzyme of DNA synthesis, ribonucleotide reductase. It takes part in myelin generation and mitochondrial energy metabolism in the brain (Kell 2009). Neuronal development and the generation of complex neural network need adequate energy supply. Mitochondria, the energy house of cells, play significant role in the development of Fe-S clusters. Fe-S clusters are essential for regulating neural functions by affecting secondary signaling cascades and neurotransmitter release (Burgoyne 2007). Different brain regions contain varied concentrations of iron depending upon the function of a particular region (Koeppen 1995; Zecca et al. 2004). Neuronal iron uptake by DMT1 has been found to be essential for normal hippocampal neuronal development (Carlson et al. 2009). Although iron is available throughout the CNS at varied concentrations, it primarily affects the genesis and

activity of the hippocampal neurons as this segment is responsible for learning and memory processes (Hidalgo and Núñez 2007; Fretham et al. 2011). Iron plays important role in normal development of cognitive functions (Hidalgo and Núñez 2007; Fretham et al. 2011). Iron deficiency in early life alters the neurochemical profile of hippocampal neurons to cause irreversible impairments of learning and memory (Lozoff 2011; Rao et al. 2003). The correlation between alterations in synaptic plasticity due to iron deficiency and cognitive impairment has been observed in several studies with rodent models (McEchron et al. 2005; Munoz et al. 2011). A reduction in prolyl hydroxylase activity results in uncontrolled HIF1α expression and translocation to the nucleus to cause an overexpression of HIF-targeted genes leading to metabolic dysregulation and neuronal dysfunction (Siddiq et al. 2008; Trollmann and Gassmann 2009; Hu et al. 2010).

Neuronal iron homeostasis is tightly regulated by several sets of iron-related proteins which include divalent metal transporter 1 (DMT1) and transferrin receptor 1 for iron uptake, ferroportin for iron export, and ferritin heavy (-H) and light (-L) chains for iron storage (Rouault 2006; Dev et al. 2015). Neurons primarily express ferritin-H to store intracellular iron (Zecca et al. 2004). Generally, ferritin-H is found in cells that utilize high amount of iron with lesser need of storage (Levi et al. 1992; Connor et al. 1994; Dev et al. 2015). Neuromelanin is also suggested as another important molecule for the sequestration of cytosolic iron in neurons (Zecca et al. 1996).

Iron accumulation in the brain is an age-dependent process (Koeppen 1995, 2003). High concentration of iron is observed in those areas associated with motor functions (Koeppen 2003). Histopathological studies have indicated that the increase in ferritin-bound iron concentration in these regions takes place during the first three decades of life that may further increase with aging (Zecca et al. 2004; Hallgren and Sourander 1958). Iron accumulated during adulthood is predominantly the non-transferrin bound form that is highly reactive and consequently generates hydroxyl radicals when present beyond the cellular threshold level. Therefore, neurons need tightly regulated iron homeostasis in order to prevent themselves from ROS-induced oxidative injury. Excess iron accumulation is detected in Alzheimer disease's (AD), Parkinson disease's (PD), and in almost all neurodegenerative diseases.

4 Iron Homeostasis in Astroglia

The presence of astrocyte in the vicinity of synapses helps them to regulate the neuronal network (Bellot-Saez et al. 2017). Astrocytes have end feet covering the abluminal surface of capillary endothelial cells (Dringen et al. 2007). They can act as gatekeepers to restrict the entry of ion from the circulation to the neuropil. Secondly, during iron overload, most of the non-transferrin bound iron circulates freely in CNS and may pose a threat to neuronal survival. Astrocytes play significant roles in storing this free iron pool to buffer the changes in iron concentration, thereby

preventing oxidative stress (Pelizzoni et al. 2013). These facts render astrocytes as players of paramount importance in regulating the iron balance of CNS.

Reports indicate that astrocytes do not express transferrin receptor in vivo (Moos and Morgan 2004; Jeong and David 2006). The expression of transferrin receptor in in vitro astrocyte cultures depends on local iron load (Bishop and Robinson 2001). Several lines of evidence recently showed that astrocytes can take up non-transferrin bound iron by DMT1; however its expression is being confined to the perivascular end feet in the CNS (Lane et al. 2010; Moos and Morgan 2004a). This area-specific expression of DMT1 may be due to the contact of astrocyte end feet with capillary endothelial cell surface to facilitate the intake of non-transferrin bound iron. This theory fits well with the fact that before being available to the astrocytes, the capillary endothelial separates iron from transferrin (Moos et al. 2006). DMT1 is specifically responsible for importing Fe^{2+} into the astrocytes. Surprisingly, studies with cultured astrocytes indicate their high capability in taking up iron supplied in the ferric form. This necessitates one to assume the presence of a ferric reductase to act in tandem with DMT1 on astrocyte membrane although no direct evidence in its support has been provided yet. The role of zinc transporter Zip14 in iron uptake by astrocytes has also been reported (Bishop et al. 2010).

Astrocytes account for very much less amount of iron storage in a normal brain, but the storage capacity gradually increases as a factor of age (Connor et al. 1990). Recent study in rat brain reported the presence of higher iron in glial cells than neurons as detected by nuclear microprobe and scanning proton-induced X-ray emission microscopy (μPIXE) (Reinert et al. 2019). This in situ measurement revealed that iron concentration in neocortical oligodendrocytes and astrocytes was fivefold and twofold higher, respectively, compared to neurons (Reinert et al. 2019). Some populations of astrocytes stain positively for ferritin in the gray matter of basal ganglia. The ferritin levels in cultured astrocytes are posttranscriptionally regulated by iron (Regan et al. 2002). Application of iron chelators results in a decrease in ferritin levels in astrocytes, whereas heme supplementation increases ferritin level (Hoepken et al. 2004). As the regulation of astrocytic ferritin expression is highly dependent on the availability of free iron pool, hemin must be accumulated and metabolized within astrocytes. Inducible heme oxygenase 1 (HO-1) degrades heme in the cytosol to generate free iron. The strong expression of HO-1 by astrocytes in normal and diseased conditions suggests the role of astrocytes in heme metabolism (Ham and Schipper 2000; Schipper 2004). The iron derived from heme may be toxic to the neurons. Hence its metabolism inside the astrocytes may be a protective measure of the CNS. Hypoxia and ischemia-reperfusion leads to an increase in astrocytic ferritin levels, which supports the fact that increased expression of ferritin acts as cytoprotectant to decrease the ROS generation by increasing the storage of free iron (Regan et al. 2002).

The export of iron in astrocytes is primarily mediated by ferroportin like all other mammalian cells. Ferroportin exports Fe^{2+} out of the cells; the Fe^{2+} is then converted into Fe^{3+} by ceruloplasmin present in astrocyte membrane. Interestingly, the ferroxidase activity of ~~ca~~eruloplasmin is important also in stabilizing ferroportin as ceruloplasmin deficiency has been reported to impair the iron export in astrocytes

(Patel and David 1997; Lawen and Lane 2013; Tapryal et al. 2009). Addition of soluble ceruloplasmin restores iron export in genetically ceruloplasmin-deficient astrocytes confirming its requirement in iron release from astrocytes (Jeong and David 2003). Recent evidence also suggests the capacity of astrocytes in synthesizing secretory ceruloplasmin (McCarthy and Kosman 2014).

5 Role of Astrocytes in Neuronal Iron Homeostasis

The free iron pool is harmful to neuronal survival as it catalyzes the Fenton-type reaction leading to ROS generation worsening the condition of CNS components during neurodegeneration. Surprisingly, CNS needs iron-mediated ROS production for long-term potentiation and basal synaptic transmission (Munoz et al. 2011). Therefore, the entry of free iron into neurons is a double-edged sword as its imbalance is likely to be highly detrimental (Codazzi et al. 2015). Acute brain injury and neurodegeneration induce DMT1 expression in neurons to facilitate the entry of free iron (Huang et al. 2006; Wang et al. 2010). The situation worsens further when iron enters neuronal cells through Ca^{2+} permeable channels (Hentze et al. 2010).

Astrocytes store iron within ferritin and release iron through ferritinophagy when requited. Therefore, these cells may be evolutionarily responsible for iron trafficking to neurons (Dringen et al. 2007). Mutation in ceruloplasmin gene resulting in decreased expression of the protein is associated with increased iron accumulation in astrocytes (Kaneko et al. 2002). In conditions of iron deprivation, astrocytes release soluble ceruloplasmin that facilitates the entry of iron into neurons (Ke et al. 2006). Astrocytes, owing to their high level of antioxidant capacity, are usually resistant to oxidative stress (Pelizzoni et al. 2011). The transcription factor Nrf2 plays the central role in this defense mechanism (Vargas and Johnson 2010). This property makes them suitable for buffering iron overload at the perisynaptic junctions by taking it up via transient receptor potential canonical channels (Codazzi et al. 2015). Pathological conditions including neurodegenerative disorders induce DMT1 expression in neurons to favor iron overload. Interestingly, high DMT1 expression in these conditions has been observed in astrocytes, in order to make them more efficient in iron buffering (Xiong et al. 2008; Zarruk et al. 2015).

Brain iron pool increases with aging, and the free iron is selectively accumulated in astrocytes with high storage capacity due to increased ferritin expression with concurrent decrease in their iron export potential due to decreased level of ceruloplasmin (Connor et al. 1990; Jeong and David 2006). These results may strengthen the theory regarding the role of astrocytes in buffering neuronal iron pool.

6 Dysregulation of Iron Homeostasis During Neurodegeneration: Correlation with Astrocyte Function

Iron participates in oxidation-reduction reaction owing to its two oxidation states that allow the interconversion between divalent (Fe^{2+}) and trivalent state (Fe^{3+}) (Papanikolaou and Pantopoulos 2005). This may lead to the generation of free radicals specially when excess free iron is present in the brain. Iron-induced oxidative damage has been observed in several neurological disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) (Moos and Morgan 2004; Haider 2015). Brain iron accumulation increases with age with concurrent increase of ferritin (Zecca et al. 2004; Connor et al. 1990). In aged brains, the ferritin bound soluble iron may be converted into hemosiderin and other oxyhydroxides with higher reactivity of iron (Crichton and Pierre 2001). Acidic conditions may lead to the release of iron from transferrin and ferritin (Bishop and Robinson 2001). In condition like poor endogenous antioxidative defense in the brain, trace amount of iron accumulation may lead to pathogenic conditions (Alexandrov et al. 2005). Iron-deficient cells have been suggested to be resistant to oxidative damage (Arosio and Levi 2002). However, iron-induced lipid peroxidation has been reported to cause apoptosis in neuronal cells (Zhang et al. 2003). Studies with rat hippocampal neurons have found that iron chelators could attenuate iron-induced free radical generation (Zhang et al. 1993). Other studies indicated the mitochondrial respiratory chain machinery of neuronal cells as the prime target of iron-induced oxidative stress (Calabrese et al. 2005; Shamoto-Nagai et al. 2006). Systemic iron levels do not correlate with iron accumulation within the CNS (Schenck and Zimmerman 2004). Thus, brain iron overload may originate from the malfunction of neuronal iron homeostasis machinery and subsequent housekeeping cellular responses. However, there are certain neurodegenerative disorders like aceruloplasminemia and neuroferritinopathy, in which mutations in ceruloplasmin and ferritin-L genes, respectively, result in brain iron accumulation primarily in astrocytes and neurons (Kaneko et al. 2002; Dusek et al. 2012; Rouault 2013). Also, there are other conditions such as Parkinson's disease (PD) and aging where iron accumulation has been reported in astroglial cells as well (Jellinger et al. 1990; Connor et al. 1990).

The retrograde degradation of dopaminergic neurons in PD has been linked with an increase in iron content in regions like substantia nigra and medial globus pallidus (Dexter et al. 1991; Griffiths et al. 1999). Ferritin shows a high iron load in PD (Griffiths et al. 1999). The cytosolic iron is sequestered by neuromelanin that can activate microglia leading to the release of proinflammatory cytokines to cause subsequent neuronal death (Langston et al. 1999; Wilms et al. 2003). There are evidences of high iron accumulation leading to oxidative stress-induced lipid peroxidation promoting the autoxidation of dopamine resulting in disruption of dopamine metabolism followed by neurodegeneration (Mohankumar et al. 1994; Sziráki et al. 1998). Also, iron-induced ROS production leads to α-synuclein aggregation leading to the generation of advanced glycation end products (Münch et al. 2000). Recent studies suggest that the available labile iron pool (LIP) may regulate α-synuclein

expression (Song et al. 2018). α-synuclein is a cellular ferrireductase that converts Fe^{3+} to bioavailable Fe^{2+} (Brown 2013).

The role of astrocytes in PD is not clearly understood so far despite report of iron accumulation in the diseased condition. Reactive astroglia that protects neurons during diseased conditions is reported to be absent in autopsy sample of substantia nigra of PD patients (Mirza et al. 2000). A strong correlation between astroglial accumulation of α-synuclein and the progression of PD has been drawn from in vitro and in vivo studies (Gu et al. 2010; Lee et al. 2010; McGann et al. 2012). Several PD-related genes are expressed in astrocytes, at levels comparable to and sometimes even higher than neurons. There are eight PD-related genes, which have been reported to have a role in astrocyte biology (Booth et al. 2017).

Astroglia play pivotal role in maintaining iron homeostasis of the CNS as an integral component of the blood-brain barrier. Ceruloplasmin, expressed in astroglia, helps in converting Fe^{2+} to Fe^{3+} by virtue of its ferroxidase capacity to facilitate cellular iron release. The presence of astroglial ceruloplasmin may help in regulating neuronal iron balance (Wang et al. 2007; Burkhart et al. 2016; Song et al. 2018). Astrocytes play active roles in the survival of dopaminergic neurons (Saura et al. 2003). The heme oxygenase-1 in astrocytes that is overexpressed in PD may play a key role in neuroprotection (Xu et al. 2016). The astroglial accumulation and subsequent degradation of α-synuclein is another mode of neuroprotection by the astrocytes unless the accumulation exceeds certain threshold levels (Lindstrom et al. 2017). Reactive astroglia produced during diseased state may also confer neuroprotection by limiting the spread of drastic effects within the CNS; however, uncontrolled or prolonged reactive astrogliosis may lead to neurodegeneration (Glass et al. 2010; Pyatigorskaya et al. 2014). Astrocytes maintain their own iron balance by modulating the iron uptake, export, and storage components (Hoepken et al. 2004; Oshiro et al. 2008). They possess iron exporter ferroportin along with the ferroxidase ceruloplasmin to make them a suitable candidate for efficient iron export. Moreover, the astrocytic end feet expresses DMT1 to take up and redistribution of brain iron (Erikson and Aschner 2006). Therefore, astrocytes can help in reducing the iron load in neurons.

The brain of the Alzheimer's patients has been detected with large amount of iron accumulation along with senile plaques and neurofibrillary tangles, whereas increase in ferritin was not found, suggesting increased risk of oxidative stress by intracellular free iron pool (Bishop et al. 2002; Castellani et al. 1999). Iron has direct effect on amyloid precursor protein processing by modulating α-secretase activity (Rogers et al. 2002; Bodovitz et al. 1995). Moreover, promotion of the deposition of amyloid β plaques was found by iron (Mantyh et al. 1993). A direct role of iron on promoting AD has been confirmed from the observation on patients with hemochromatosis as they tend to develop AD at an earlier stage than the non-hemochromatosis patients (Núñez-Millacura et al. 2002).

The precise role of astrocytes in AD, however, is not clear so far. Numerous reports have shown that reactive astrogliosis during AD leads to neurodegenerative process by secreting pro-inflammatory cytokines (Sajja et al. 2016). On the other hand, reactive astrocytes were reported to play a role in the clearance of amyloid β

plaques by secreting matrix metalloproteinases and increasing expression of neprilysin and insulin-degrading enzyme, thereby conferring protection to the neurons during AD (Yin et al. 2006; Liu et al. 2016; Fakhoury 2018). Although several reports strongly indicated a correlation among astrocyte function, iron metabolism, and neurodegeneration, elaborative research on understanding the precise role of astrocytes in controlling iron metabolism during the neurodegenerative events like AD is still under progress.

7 Future Perspectives

The role of astrocytes in iron homeostasis and maintaining the neuronal iron balance has not been fully understood. Detailed studies with in vivo models are necessary to elucidate the precise role of astrocytes in neuronal iron homeostasis. Recent therapeutic approaches in the neurodegeneration field include the targeting of astrocytic Nrf2 pathway by modulation with exogenous antioxidants for conferring better protection against neurons. Interestingly a recent study showed that astrocytes exposed to cytokines undergo "activation" conferring a protective effect by primarily expressing high amount of SOD2, and this mechanism was independent of Nrf2 pathway (Macco et al. 2013). Practically, elucidation of molecular interactions in astrocytic iron homeostasis is a herculean task. Genetically modified animal, gene silencing techniques, along with various omics analysis may clarify the inner core of such complex network. As astrocyte activation plays major role in neuronal fate, modulation of the same may help in neuroprotection, provided the specific interactions are well understood. In addition, astrocytes may act like a double-edged sword, i.e., to supply iron to neurons for normal synaptic functioning, and to limit the iron supply to avoid its detrimental effects. Existing reports indicate that astrocyte may have a neuroprotective role up to a certain stage of neurodegeneration after which its own homeostasis is perturbed resulting in toxic neurodegenerative effects. A detailed understanding of the interaction of astrocytes with neurons in relation to iron homeostasis may pave the way to target astrocytes for therapeutic strategies in order to confer protection against iron-induced neurodegeneration. In this context, elucidating the role of astrocyte-astrocyte communication in maintaining synaptic signaling will be an important aspect to investigate.

Acknowledgement CKM acknowledges financial support received from National Initiative on Glial Cell Research in Health and Disease from Department of Biotechnology and SERB-Department of Science and Technology, India.

References

Alexandrov PN, Zhao Y, Pogue AI et al (2005) Synergistic effects of iron and aluminium on stress-related gene expression in primary human neural cells. J Alzheimers Dis 8:117–127

Arosio P, Levi S (2002) Ferritin, iron homeostasis, and oxidative damage. Free Radic Biol Med 33: 457–463

Belanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab 14:724–738

Bellot-Saez A, Kékesi O, Morley JW, Buskila Y (2017) Astrocytic modulation of neuronal excitability through K^+ spatial buffering. Neurosci Biobehav Rev 77:87–97

Bishop GM, Robinson SR (2001) Quantitative analysis of cell death and ferritin expression in response to cortical iron: implications for hypoxia-ischemia and stroke. Brain Res 907:175–187

Bishop GM, Robinson SR, Liu Q, Perry G, Atwood CS, Smith MA (2002) Iron: a pathological mediator of Alzheimer disease? Dev Neurosci 24:184–187

Bishop GM, Scheiber IF, Dringen R, Robinson SR (2010) Synergistic accumulation of iron and zinc by cultured astrocytes. J Neural Transm 117:809–817

Bodovitz S, Falduto MT, Frail DE, Klein WL (1995) Iron levels modulate alpha-secretase cleavage of amyloid precursor protein. J Neurochem 64:307–315

Booth HDE, Hirst WD, Wade-Martins R (2017) The role of astrocyte dysfunction in Parkinson's disease pathogenesis. Trends Neurosci 40:358–370

Brambilla R, Morton PD, Ashbaugh JJ, Karmally S, Lambertsen KL, Bethea JR (2014) Astrocytes play a key role in EAE pathophysiology by orchestrating in the CNS the inflammatory response of resident and peripheral immune cells and by suppressing remyelination. Glia 62:452–467

Brown DR (2013) α-Synuclein as a ferrireductase. Biochem Soc Trans 41:1513–1517

Burgoyne RD (2007) Neuronal calcium sensor proteins: generating diversity in neuronal Ca^{2+} signalling. Nat Rev Neurosci 8:182–193

Burkhart A, Skjorringe T, Johnsen KB et al (2016) Expression of iron-related proteins at the neurovascular unit supports reduction and reoxidation of iron for transport through the blood-brain barrier. Mol Neurobiol 53:7237–7253

Buskila Y, Morley JW, Tapson J, van Schaik A (2013) The adaptation of spike back propagation delays in cortical neurons. Front Cell Neurosci 7:192

Buskila Y, Bellot-Saez A, Morley JW (2019) Generating brain waves, the power of astrocytes. Front Neurosci 13:1125

Calabrese V, Lodi R, Tonon C et al (2005) Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia. J Neurol Sci 233:145–162

Carlson ES, Tkac I, Magid R et al (2009) Iron is essential for neuron development and memory function in mouse hippocampus. J Nutr 139:672–679

Castellani RJ, Perry G, Brenner DS, Smith MA (1999) Alexander disease: Alzheimer disease of the developing brain? Alzheimer Dis Assoc Disord 13:232–235

Codazzi F, Pelizzoni I, Zacchetti D, Grohovaz F (2015) Iron entry in neurons and astrocytes: a link with synaptic activity. Front Mol Neurosci 8:18

Connor JR, Menzies SL, St Martin SM, Mufson EJ (1990) Cellular distribution of transferrin, ferritin, and iron in normal and aged human brains. J Neurosci Res 27:595–611

Connor JR, Boeshore KL, Benkovic SA, Menzies SL (1994) Isoforms of ferritin have a specific cellular distribution in the brain. J Neurosci Res 37:461–465

Crichton RR, Pierre JL (2001) Old iron, young copper: from Mars to Venus. Biometals 14:99–112

Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1–105

Dev S, Kumari S, Singh N, Bal SK, Seth P, Mukhopadhyay CK (2015) Role of extracellular hydrogen peroxide on regulation of iron homeostasis genes in neuronal cell: implication in iron accumulation. Free Radic Biol Med 86:78–89

Dexter DT, Carayon A, Javoy-Agid F et al (1991) Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. Brain 114:1953–1975

Ding F, O'Donnell J, Xu Q et al (2016) Changes in the composition of brain interstitial ions control the sleep-wake cycle. Science 352:550–555

Dringen R, Bishop GM, Koeppe M, Dang TN, Robinson SR (2007) The pivotal role of astrocytes in the metabolism of iron in the brain. Neurochem Res 32:1884–1890

Dusek P, Jankovic J, Le W (2012) Iron dysregulation in movement disorders. Neurobiol Dis 46:1–18

Erikson KM, Aschner M (2006) Increased manganese uptake by primary astrocyte cultures with altered iron status is mediated primarily by divalent metal transporter. Neurotoxicology 27:125–130

Fakhoury M (2018) Microglia and astrocytes in Alzheimer's disease: implications for therapy. Curr Neuropharmacol 16:508–518

Fretham SJ, Carlson ES, Georgieff MK (2011) The role of iron in learning and memory. Adv Nutr 2:112–121

Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH (2010) Mechanisms underlying inflammation in neurodegeneration. Cell 140:918–934

Griffiths PD, Dobson BR, Jones GR, Clarke DT (1999) Iron in the basal ganglia in Parkinson's disease: an in vitro study using extended X-ray absorption fine structure and cryo-electron microscopy. Brain 122:667–673

Gu XL, Long CX, Sun L, Xie C, Lin X, Cai H (2010) Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. Mol Brain 3:12

Haider L (2015) Inflammation, iron, energy failure, and oxidative stress in the pathogenesis of multiple sclerosis. Oxidative Med Cell Longev 2015:725370

Hallgren B, Sourander P (1958) The effect of age on the non-haemin iron in the human brain. J Neurochem 3:41–51

Ham D, Schipper HM (2000) Heme oxygenase-1 induction and mitochondrial iron sequestration in astroglia exposed to amyloid peptides. Cell Mol Biol (Noisy-le-Grand) 46:587–596

Hentze MW, Muckenthaler MU, Galy B, Camaschella C (2010) Two to tango: regulation of mammalian iron metabolism. Cell 142:24–38

Hidalgo C, Núñez MT (2007) Calcium, iron and neuronal function. IUBMB Life 59:280–285

Hoepken HH, Korten T, Robinson SR, Dringen R (2004) Iron accumulation, iron-mediated toxicity and altered levels of ferritin and transferrin receptor in cultured astrocytes during incubation with ferric ammonium citrate. J Neurochem 88:1194–1202

Hu Z, Gulec S, Collins JF (2010) Cross-species comparison of genomewide gene expression profiles reveals induction of hypoxia-inducible factor-responsive genes in iron-deprived intestinal epithelial cells. Am J Physiol Cell Physiol 299:C930–C938

Huang E, Ong WY, Go ML, Connor JR (2006) Upregulation of iron regulatory proteins and divalent metal transporter-1 isoforms in the rat hippocampus after kainate induced neuronal injury. Exp Brain Res 170:376–386

Jellinger K, Paulus W, Grundke-Iqbal I, Riederer P, Youdim MB (1990) Brain iron and ferritin in Parkinson's and Alzheimer's diseases. J Neural Transm Park Dis Dement Sect 2:327–340

Jeong SY, David S (2003) Glycosylphosphatidylinositol-anchored ceruloplasmin is required for iron efflux from cells in the central nervous system. J Biol Chem 278:27144–27148

Jeong SY, David S (2006) Age-related changes in iron homeostasis and cell death in the cerebellum of ceruloplasmin deficient mice. J Neurosci 26:9810–9819

Kaneko K, Yoshida K, Arima K, Ohara S, Miyajima H, Kato T, Ohta M, Ikeda SI (2002) Astrocytic deformity and globular structures are characteristic of the brains of patients with aceruloplasminemia. J Neuropathol Exp Neurol 61:1069–1077

Ke Y, Ho K, Du J et al (2006) Role of soluble ceruloplasmin in iron uptake by midbrain and hippocampus neurons. J Cell Biochem 98:912–919

Kell DB (2009) Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. BMC Med Genet 2:2

Koeppen AH (1995) The history of iron in the brain. J Neurol Sci 134(Suppl):1–9

Koeppen AH (2003) A brief history of brain iron research. J Neurol Sci 207:95–97

Lane DJ, Robinson SR, Czerwinska H, Bishop GM, Lawen A (2010) Two routes of iron accumulation in astrocytes: ascorbate-dependent ferrous iron uptake via the divalent metal transporter (DMT1) plus an independent route for ferric iron. Biochem J 432:123–132

Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D (1999) Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. Ann Neurol 46:598–605

Laudanski J, Torben-Nielsen B, Segev I, Shamma S (2014) Spatially distributed dendritic resonance selectively filters synaptic input. PLoS Comput Biol 10(8):e1003775

Lawen A, Lane DJ (2013) Mammalian iron homeostasis in health and disease: uptake, storage, transport, and molecular mechanisms of action. Antioxid Redox Signal 18:2473–2507

Lee HJ, Suk JE, Patrick C et al (2010) Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. J Biol Chem 285:9262–9272

Levi S, Yewdall SJ, Harrison PM et al (1992) Evidence of H- and L- chains have cooperative roles in the iron-uptake mechanism of human ferritin. Biochem J 288:591–596

Lindstrom V, Gustafsson G, Sanders LH et al (2017) Extensive uptake of α-synuclein oligomers in astrocytes results in sustained intracellular deposits and mitochondrial damage. Mol Cell Neurosci 82:143–156

Liu RX, Huang C, Bennett DA, Li H, Wang R (2016) The characteristics of astrocyte on Aβ clearance altered in Alzheimer's disease were reversed by anti-inflammatory agent (+)-2-(1-hydroxyl-4oxocyclohexyl) ethyl caffeate. Am J Transl Res 8:4082–4094

Lozoff B (2011) Early iron deficiency has brain and behavior effects consistent with dopaminergic dysfunction. J Nutr 141:740S–746S

Ma Z, Stork T, Bergles DE, Freeman MR (2016) Neuromodulators signal through astrocytes to alter neural circuit activity and behavior. Nature 539:428–432

Macco R, Pelizzoni I, Consonni A et al (2013) Astrocytes acquire resistance to iron-dependent oxidative stress upon proinflammatory activation. J Neuroinflammation 10:130

Mantyh PW, Ghilardi JR, Rogers S et al (1993) Aluminum, iron, and zinc ions promote aggregation of physiological concentrations of beta-amyloid peptide. J Neurochem 61:1171–1174

Maragakis NJ, Rothstein JD (2006) Mechanism of disease: astrocytes in neurodegenerative disease. Nat Clin Pract Neurol 2:679–689

Matias I, Morgado J, Gomes FCA (2019) Astrocyte heterogeneity: impact to brain aging and disease. Front Aging Neurosci 11:59

McCarthy RC, Kosman DJ (2014) Glial cell ceruloplasmin and hepcidin differentially regulate iron efflux from brain microvascular endothelial cells. PLoS One 9(2):e89003

McEchron MD, Cheng AY, Liu H, Connor JR, Gilmartin MR (2005) Perinatal nutritional iron deficiency permanently impairs hippocampus-dependent trace fear conditioning in rats. Nutr Neurosci 8:195–206

McGann JC, Lioy DT, Mandel G (2012) Astrocytes conspire with neurons during progression of neurological disease. Curr Opin Neurobiol 22:850–858

Mirza B, Hadberg H, Thomsen P, Moos T (2000) The absence of reactive astrocytosis is indicative of a unique inflammatory process in Parkinson's disease. Neuroscience 95:425–432

Mohankumar KP, De Bartolomeis A, Wu RM et al (1994) Ferrous-citrate complex and nigral degeneration: evidence for free-radical formation and lipid peroxidation. Ann N Y Acad Sci 738:392–399

Moos T, Morgan EH (2004) The metabolism of neuronal iron and its pathogenic role in neurological disease: review. Ann N Y Acad Sci 1012:14–26

Moos T, Morgan EH (2004a) The significance of the mutated divalent metal transporter (DMT1) on iron transport into the Belgrade rat brain. J Neurochem 88:233–245

Moos T, Skjoerringe T, Gosk S, Morgan EH (2006) Brain capillary endothelial cells mediate iron transport into the brain by segregating iron from transferrin without the involvement of divalent metal transporter 1. J Neurochem 98:1946–1958

Mukhopadhyay CK, Dev S, Tapryal N, Mukherjee R, Mukhopadhayay C (2011) Role of redox and ceruloplasmin in iron deposition in glial cells: implication in neurodegenerative damages. IIOAB J 2:1–8

Münch G, Lüth HJ, Wong A et al (2000) Crosslinking of alpha-synuclein by advanced glycation endproducts—an early pathophysiological step in Lewy body formation? J Chem Neuroanat 20: 253–257
Munoz M, Garcia-Erce JA, Remacha AF (2011) Disorders of iron metabolism. Part II: Iron deficiency and iron overload. J Clin Pathol 64:287–296
Núñez-Millacura C, Tapia V, Muñoz P, Maccioni RB, Núñez MT (2002) An oxidative stress-mediated positive-feedback iron uptake loop in neuronal cells. J Neurochem 82:240–248
Oshiro S, Kawamura K, Zhang C et al (2008) Microglia and astroglia prevent oxidative stress-induced neuronal cell death: implications for aceruloplasminemia. Biochim Biophys Acta 1782: 109–117
Papanikolaou G, Pantopoulos K (2005) Iron metabolism and toxicity. Toxicol Appl Pharmacol 202: 192–211
Patel BN, David S (1997) A novel glycosylphosphatidylinositol-anchored form of ceruloplasmin is expressed by mammalian astrocytes. J Biol Chem 272:20185–20190
Pelizzoni I, Macco R, Morini MF, Zacchetti D, Grohovaz F, Codazzi F (2011) Iron handling in hippocampal neurons: activity-dependent iron entry and mitochondria-mediated neurotoxicity. Aging Cell 10:172–183
Pelizzoni I, Zacchetti D, Campanella A, Grohovaz F, Codazzi F (2013) Iron uptake in quiescent and inflammation-activated astrocytes: a potentially neuroprotective control of iron burden. Biochim Biophys Acta 1832:1326–1333
Perea G, Navarrete M, Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32:421–431
Pyatigorskaya N, Gallea C, Garcia-Lorenzo D, Vidailhet M, Lehericy S (2014) A review of the use of magnetic resonance imaging in Parkinson's disease. Ther Adv Neurol Disord 7:206–220
Rao R, Tkac I, Townsend EL, Gruetter R, Georgieff MK (2003) Perinatal iron deficiency alters the neurochemical profile of the developing rat hippocampus. J Nutr 133:3215–3221
Regan RF, Kumar N, Gao F, Guo Y (2002) Ferritin induction protects cortical astrocytes from heme-mediated oxidative injury. Neuroscience 113:985–994
Reinert A, Morawski M, Seeger J, Arendt T, Reinert T (2019) Iron concentrations in neurons and glial cells with estimates on ferritin concentrations. BMC Neurosci 20:25
Rogers JT, Randall JD, Cahill CM et al (2002) An iron-responsive element type II in the 5-′-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem 277:45518–45528
Rouault TA (2006) The role of iron regulatory proteins in mammalian iron homeostasis and disease. Nat Chem Biol 2:406–414
Rouault TA (2013) Iron metabolism in the CNS: implications for neurodegenerative diseases. Nat Rev Neurosci 14:551–564
Sajja VS, Hlavac N, VandeVord PJ (2016) Role of glia in memory deficits following traumatic brain injury: biomarkers of glia dysfunction. Front Integr Neurosci 10:7
Saura J, Pares M, Bove J et al (2003) Intranigral infusion of interleukin-1 beta activates astrocytes and protects from subsequent 6-hydroxydopamine neurotoxicity. J Neurochem 85:651–661
Schenck JF, Zimmerman EA (2004) High-field magnetic resonance imaging of brain iron: birth of a biomarker? NMR Biomed 17:433–445
Schipper HM (2004) Heme oxygenase expression in human central nervous system disorders. Free Radic Biol Med 37:1995–2011
Shamoto-Nagai M, Maruyama W, Yi H et al (2006) Neuromelanin induces oxidative stress in mitochondria through release of iron: mechanism behind the inhibition of 26S proteasome. J Neural Transm (Vienna) 113:633–644
Siddiq A, Aminova LR, Ratan RR (2008) Prolyl 4-hydroxylase activity responsive transcription factors: from hydroxylation to gene expression and neuroprotection. Front Biosci 13:2875–2887
Simard M, Nedergaard M (2004) The neurobiology of glia in the context of water and ion homeostasis. Neuroscience 129:877–896

Singh N, Haldar S, Tripathi AK, Horback K et al (2014) Brain iron homeostasis: from molecular mechanisms to clinical significance and therapeutic opportunities. Antioxid Redox Signal 20: 1324–1363

Snyder SH (2011) What dopamine does in the brain. Proc Natl Acad Sci U S A 108:18869–18871

Song N, Wang J, Jiang H, Xie J (2018) Astroglial and microglial contributions to iron metabolism disturbance in Parkinson's disease. Biochim Biophys Acta Mol basis Dis 1864:967–973

Svichar N, Chesler M (2003) Surface carbonic anhydrase activity on astrocytes and neurons facilitates lactate transport. Glia 41:415–419

Sziráki I, Mohanakumar KP, Rauhala P, Kim HG, Yeh KJ, Chiueh CC (1998) Manganese: a transition metal protects nigrostriatal neurons from oxidative stress in the iron-induced animal model of parkinsonism. Neuroscience 85:1101–1111

Takata N, Hirase H (2008) Cortical layer 1 and layer 2/3 astrocytes exhibit distinct calcium dynamics in vivo. PLoS One 3:e2525

Tanaka K, Watase K, Manabe T et al (1997) Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 276:1699–1702

Tapryal N, Mukhopadhyay C, Das D, Fox PL, Mukhopadhyay CK (2009) Reactive oxygen species regulate ceruloplasmin by a novel mRNA decay mechanism involving its 3′-untranslated region: implications in neurodegenerative diseases. J Biol Chem 284:1873–1883

Trollmann R, Gassmann M (2009) The role of hypoxia-inducible transcription factors in the hypoxic neonatal brain. Brain Dev 31:503–509

Vargas MR, Johnson JA (2010) Astrogliosis in amyotrophic lateral sclerosis: role and therapeutic potential of astrocytes. Neurotherapeutics 7:471–481

Verkhratsky A, Nedergaard M (2018) Physiology of astroglia. Physiol Rev 98:239–389

Wang J, Jiang H, Xie JX (2007) Ferroportin1 and hephaestin are involved in the nigral iron accumulation of 6-OHDA-lesioned rats. Eur J Neurosci 25:2766–2772

Wang D, Wang L-H, Zhao Y, Lu Y-P, Zhu L (2010) Hypoxia regulates the ferrous iron uptake and reactive oxygen species level via divalent metal transporter 1 (DMT1) Exon1B by hypoxia inducible factor-1. IUBMB Life 62:629–636

Wilms H, Rosenstiel P, Sievers J, Deuschl G, Zecca L, Lucius R (2003) Activation of microglia by human neuromelanin is NF-kappaB dependent and involves p38 mitogen-activated protein kinase: implications for Parkinson's disease. FASEB J 17:500–502

Xiong ZG, Pignataro G, Li M, Chang SY, Simon RP (2008) Acid sensing ion channels (ASICs) as pharmacological targets for neurodegenerative diseases. Curr Opin Pharmacol 8:25–32

Xu X, Song N, Wang R, Jiang H, Xie J (2016) Preferential heme oxygenase-1 activation in striatal astrocytes antagonizes dopaminergic neuron degeneration in MPTP-intoxicated mice. Mol Neurobiol 53:5056–5065

Yin K-J, Cirrito JR, Yan P et al (2006) Matrix metalloproteinases expressed by astrocytes mediate extracellular amyloid-beta peptide catabolism. J Neurosci 26:10939–10948

Zarruk JG, Berard JL, Santos PDR et al (2015) Expression of iron homeostasis proteins in the spinal cord in experimental autoimmune encephalomyelitis and their implications for iron accumulation. Neurobiol Dis 81:93–107

Zecca L, Shima T, Stroppolo A et al (1996) Interaction of neuromelanin and iron in substantia nigra and other areas of human brain. Neuroscience 73:407–415

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR (2004) Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 5:863–873

Zhang Y, Tatsuno T, Carney JM, Mattson MP (1993) Basic FGF, NGF, and IGFs protect hippocampal and cortical neurons against iron-induced degeneration. J Cereb Blood Flow Metab 13:378–388

Zhang Z, Wei T, Hou J, Li G, Yu S, Xin W (2003) Iron-induced oxidative damage and apoptosis in cerebellar granule cells: attenuation by tetramethylpyrazine and ferulic acid. Eur J Pharmacol 467:41–47

Glial Cells as Key Orchestrators of Neural Degeneration in Basal Ganglia Disorders

Bidisha Bhaduri and Phalguni Anand Alladi

Abstract

The congregation of nuclei in the forebrain, diencephalon and midbrain called 'basal ganglia' forms an important structure for neural signals associated with movement. The striatum is the major input nucleus, which comprises of various cellular types—medium spiny neurons and interneurons—and receives input from different cortical and subcortical structures. On the other hand, the substantia nigra pars reticulata is the prime output nucleus. Depletion of the dopamine in the striatum underlies the pathophysiology of certain disorders, mainly Parkinson's disease (PD). The glial cells are believed to be the chief regulators, the primary switches of the nervous system development, plasticity and disease progression. Both a loss of trophic and protective functions and a gain of toxic functions by glia are inculpated in the neurodegenerative processes. Studies in the past few decades, in the field of basal ganglia associated neurodegenerative diseases, have justified the rapt consideration denoted to glia. Amongst the diseases that involve the basal ganglia are PD and a select others characterized as the Parkinson's plus syndrome.

The present review covers, in brief, the studies published in last five decades, including the normal development of basal ganglia as well as the role of different glial cells in select neurodegenerative diseases like PD, multiple system atrophy (MSA), cortico-basal ganglia disorders (CBGD), progressive supranuclear palsy (PSP) and Wilson's disease (WD).

B. Bhaduri · P. A. Alladi (✉)
Department of Clinical Psychopharmacology and Neurotoxicology, National Institute of Mental Health and Neurosciences, Bengaluru, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_15

Keywords

Parkinson's disease · Parkinsonian plus syndromes · Striatum · Neurotoxins · Growth factors/cell therapy

1 Introduction

The basal ganglia comprise of intertwined nuclei in the forebrain, diencephalon and midbrain that form a large subcortical structure. The striatum develops during embryogenesis, from the basal aspect of lateral telencephalic vesicles, and is named so because of the striped appearance of the fibres passing through this region. It is often addressed as a unit, and it modulates the execution of a movement upon receiving inputs from the relevant motor and non-motor cortical areas and other structures. Its major constituent structures are the striatum, globus pallidus, subthalamic nuclei and substantia nigra (Nelson and Kreitzer 2014). The human striatum includes caudate and putamen (neostriatum) as well as nucleus accumbens (ventral striatum) that are distinct anatomical entities. In carnivores and primates, the putamen and caudate are separated by the internal capsule, whereas in rodent striatum, the putamen and caudate form a fused structure together with the internal fibres.

The striatum is the major input nucleus that receives principal excitatory inputs from two types of cortical pyramidal neurons of layer 5, of the cerebral cortex. First, those with exclusively intra-telencephalic connections are called 'IT type.' The second one projecting to the brainstem via the pyramidal tract is known as the 'PT type.' They project to the different parts of striatum, yet they are distinct in their functions, morphology and genetic composition. The IT-type neurons carry sensory and motor information to striatum, whereas PT-type neurons carry the efferent copy of motor directives (Reiner et al. 2010). The cortex sends connections to the matrix within the striatum to form the cortico-basal ganglia (BG)-thalamocortical loop. This is vital in selection of action and fine-tuning of behavioural responses over long-term (Shipp 2017).

The globus pallidus (GP) comprises two distinct compartments, namely, the globus pallidus interna (GPi) and the globus pallidus externa (GPe). The GPe and substantia nigra pars reticulata are the output nuclei of basal ganglia. The subthalamic nucleus (STN) is located ventral to the thalamus, at the junction of the diencephalon and midbrain. The neurons located within are glutamatergic and hence have excitatory properties and end extensively on several globus pallidus interna neurons. It receives inputs from the several motor-associated areas of the cortex, viz. primary motor cortex, premotor area, supplementary motor area and frontal eye fields. It receives inhibitory projections from globus pallidus externa which are GABAergic. Substantia nigra is likewise divided into two compartments: substantia nigra pars compacta (SNpc) and substantia nigra pars reticulata (SNpr). The pars compacta harbour dopaminergic neurons that fine-tune the activity of the direct pathway and indirect pathway through D1 receptor and D2 receptors, respectively.

These neurons are lost in Parkinson's disease (PD). The pars reticulata is a prime output nucleus of the basal ganglia. It is affected by the depletion of dopamine. The astrocytes are major players that elicit extemporaneous calcium activities even in normal conditions (Barat et al. 2012).

Both a loss of trophic and protective functions and a gain of toxic functions by glia are implicated in the commencement and promulgation of the neurodegenerative processes. Studies in the past few decades have meticulously charted out the role of glia in neurodegeneration within the basal ganglia. Amongst the diseases that involve the basal ganglia are PD and a select others characterized as the Parkinson's plus syndrome. Some examples of the common ones are multiple system atrophy (MSA), progressive supranuclear palsy (PSP) and dementia with Lewy body disease (DLB). The others are Pick's disease, parkinsonian-dementia complex of Guam, cortico-basal ganglionic degeneration (CBGD), frontotemporal dementia with chromosome 17, pallidonigral degeneration, Wilson's disease and a rigid variant of Huntington's disease (Mitra et al. 2003). The present review covers, in brief, the studies published in last 4–5 decades. These include those that deliberate on the normal development of basal ganglia as well as the role of glia in select neurodegenerative diseases like PD, MSA, CBGD, PSP and Wilson's disease. Major aspects of Huntington's disease and PD have been dealt with by others in the present compendium and hence have been skipped in this chapter.

2 Morphometric Studies of Glia in Normal Human Striatum

In order to understand the abnormal, it is a must to understand the normal. Several studies initially delved into the normal organization and connections of the basal ganglia structures. Amongst the earliest were the detailed morphometric and volumetric studies by anatomists, for example, Schröder et al. (1975) through a morphometric-statistical analysis on normal human adults reported that the mean numerical density of glia was 41,000 glia/mm^3, without any notable effect of gender on the numbers (7% more in males). The total number of glia decreased with age. In an interesting study, Bottcher (1975) provided the classical finding that the basal ganglia volume decreased with age and also in PD. Bøttcher (1978) further proposed that in an infant striatum, the glial distribution was random, and numbers were fewer compared to the adults. Ludwin et al. (1976) described the topographical distribution of S-100 and GFAP (glia fibrillary acidic protein), which were present in the glia limitans as well as the perivascular membranes, as also in the astroglia. S-100 was located in the nucleus as well as the cytoplasm, while GFAP was purely cytoplasmic. Its presence in the oligodendroglia confers it a general regulatory role in glia. While explaining the connection between the two proteins S100B and GFAP, Domaradzka-Pytel et al. (2000) showed that during the first postnatal week, the structure and number of GFAP-immunoreactive (ir) cells match that in adults and stabilize by P21. Using transgenic s100b-EGFP cells, Raponi et al. (2007) demonstrated that with the initiation of S100β expression, the GFAP-ir cells lose their ability to form neuro spheres and hence lose their stemness. It was therefore

associated with their maturity. Besides, the SVZ microenvironment blocked the expression of S100B, but a similar control was not available in the striatal parenchyma.

Mendell and Whitaker (1978) reported that in both CNS and PNS, the anti-myelin basic protein antibody was bound exclusively to the cellular or membranous myelin elements, thus paving the way for detection of Schwann cells and oligodendroglia. In addition to basal ganglia, Kawamoto et al. (1999) found BDNF-positive glia and neurons in the basal forebrain, cerebral cortex, hippocampus, diencephalon, brainstem and cerebellum, suggesting that glia may have a neurotrophic role.

3 Significance of Glia in Developing Basal Ganglia

Dahl (1981), in cytoskeletal preparations from newborn rat basal ganglia, showed that a large majority of cells were vimentin-ir, while a few were GFAP-ir. Vimentin was mainly localized in immature glia. It is valued as a marker till date and has applications in the field of stem cells biology (Morrow et al. 2020). Systematic studies from the Prochiantz group and others report the role of target cells using striatal and mesencephalic primary cultures. For example, Di Porzio et al. (1980) reported the importance of striatal cells for the growth of mesencephalic dopaminergic neurons including their maturation and neurite outgrowth (Denis-Donini et al. 1983; Dong et al. 1993). Mesencephalic astrocytes enhanced neuronal survival (Beyer et al. 1991) by glutathione-mediated ROS inactivation (Drukarch et al. 1998) and facilitate profuse branching of varicose neurites (Denis-Donini et al. 1984), whereas striatal glia support survival and induce the formation of a single long and linear neurite (Dong et al. 1993). Thus, glial cells have unique and regionally specific features. In addition, it was confirmed using conditioned media of striatal and nigral glia that these were releasable factors (Rousselet et al. 1990). Striatal neurons growing in presence of local glia assist the formation of synaptic targets for mesencephalic DA axons (Rouget et al. 1993).

Striatal astroglia differentiated well in vitro in the presence of substantia nigra neurons as indicated by enhanced 3H-L-glutamate uptake and GS activity (Hansson 1986). They expressed D1 dopamine receptors that probably improved the efficiency of cyclic AMP (Hansson and Rönnbäck 1988). Bal et al. (1994) using cultured rat striatal and cerebellar astrocytes showed that striatal GFAP-ir type I astroglia specifically co-expressed dopamine D2 receptor mRNA, but not cerebellar astrocytes. Thus, astrocytes are truly heterogeneous and take up region specific tasks. Interestingly, the substantia nigra neurons are responsive to olfactory bulb glia, as they show extension of neurites which remain unblocked by laminin (Denis-Donini and Estenoz 1988). Reciprocal exchange between the two regions with functional connections assumes importance in the modern understanding of PD and justifies the role of olfactory bulb and anosmia in the prodromal period.

Clonal boundaries exist within the embryonic striatum that determine the patterns of proliferation, migration and some lineage-associated relationships. Halliday and Cepko (1992) suggested that striatal progenitor cells lose stem cell pattern of

division between embryonic day 15 (E15) and E19 and produce a variety of clone types. Further, radial glia and presumptive neurons may be present within the same clone. Ling et al. (1998) reported that IL-11, leukaemia inhibitory factor, IL-1 and glial cell line-derived neurotrophic factor (GDNF) facilitate the maturation of progenitor cells from the rat foetal mesencephalon into TH-ir cells. Fragments of mesencephalic membrane and conditioned media from striatum along with the cytokines enhanced the maturation of TH-ir cells, with expression of DAT, dopa-decarboxylase and dopamine, suggesting that haematopoietic cytokines modulate the attainment of DA neuron phenotype.

Graybiel and Ragsdale (1978) drew the attention to the presence of two functionally distinct compartments in the neostriatum, i.e. the striosome and matrix. Sajin and Steindler (1994) showed that during development, these compartments are fenced by astrocytes and associated glyco-conjugates. A recent study demonstrated that the two organizational components of the striatum, i.e. the direct and indirect pathways, followed a lineage program embedded in the lateral ganglionic eminence that involved radial glial progenitors (Kelly et al. 2018). The early phase produced striosomal spiny projection neurons and the late phase produced matrix spiny projection neurons.

Basal ganglia astrocytes expressed dopamine receptors D1-, D3-, D4- and D5- and showed D4-mediated signal transduction in response to dopamine, suggesting their likely influence on the pharmacological action of atypical antipsychotic drugs and DA in some diseases (Miyazaki et al. 2004). Calbindin-D28k is a calcium-binding protein, allied with the homeostasis of intracellular calcium. It is a prominent neural protein in striatum. Liu and Graybiel (1992) demonstrated that many processes resembling the radial glia in dorsal caudo-putamen were calbindin positive. Thus, glial cells too express calbindin during development.

3.1 Astroglia and Ageing Basal Ganglia

Sturrock (1980) provided primary evidence that in ageing mouse neostriatum, the neuron/glia ratio increased between 5 and 9 months and remained steady thereafter until 22 months. The rise was attributed majorly to astrocytes and, to some extent, to oligodendrocytes. Within the neostriatum, the age-associated pigment lipofuscin was found at 6 months in astrocytes, microglia and the neighbouring ependyma and at 18 months in oligodendrocytes providing evidence of differing metabolic activity in the different glial types. In affirmation with the age effects on oligodendroglia, Sturrock (1987) demonstrated vacuole formation in myelin sheaths surrounding or within the traversing nerve fibre bundles in the aged neostriatum. Further, while oligodendrocytes show feeble evidence of ageing, satellite oligodendrocytes contained more lipofuscin.

Pasinetti et al. (1999) studied the rat striatum to assess age-associated alterations in microglia and astrocytes in two genotypes with a 35% difference in lifespans and their F1 hybrids. Even though there were differences in the lifespan, the slope of GFAP mRNA expression remained comparable. Besides GFAP mRNA was

comparatively more than other inflammation and degeneration-associated proteins like apoE, apoJ, C1q and TGF-beta1, suggesting the role of structural changes during ageing and inflammation. Morphological alterations in glia were similarly demonstrated in the ageing human substantia nigra of Asian Indians (Jyothi et al. 2015). The findings gain importance as only a mild α-synuclein aggregation occurred with age, and there was no frank loss of neurons or synaptic proteins in the nigra of ageing Asian Indians (Alladi et al. 2009, 2010a, b; Naskar et al. 2019). Howard et al. (1998) showed co-localization and age-associated accumulation of both D2R mRNA and protein in cells of neostriatum, more prominently in the medium spiny and aspiny neurons, as well as many oligodendroglia within the fibre tracts of the forebrain. They were intrigued by the expression of the dopamine D2 receptor in glial cells and explained that they may support some non-documented, non-synaptic functions.

Calorie restriction or reduced food intake assists healthy ageing in animal models. For example, in adult rodents it increases their lifespan and also attenuates age-enhanced lesions in several organs and alterations in the brain. Morgan et al. (1999) demonstrated the influence of calorie restriction on the age-associated increase in astrogliosis in basal ganglia in addition to the corpus callosum and hippocampus. In the basal ganglia a concomitant decrease in apolipoproteins J and E as well as haem oxygenase-1 was noted. Maswood et al. (2004) provided complementary findings of calorie-restricted primates having higher locomotor activity, high dopamine and metabolites levels in the striatum that resulted in better survival of nigral DA neurons and improved dexterity. Besides, the caudate nucleus showed enhanced GDNF levels, suggesting a role for calorie restrictions, glia and GDNF.

3.2 Limited Focus on Oligodendroglia

Oligodendroglia received delayed attention in basal ganglia studies, until the discovery of their relevance in multiple system atrophy. In relation to their normal development, Birling and Price (1998) proposed that multipotent precursor cells from E13 ganglionic eminence had higher capacity to generate oligodendrocytes than other cells of cerebral cortex. A combination of striatal contact and specific growth factors was essential to induce genesis of oligodendrocytes from embryonic cerebral cortex, emphasizing the role of target cells. Annese et al. (2013) demonstrated that in both mice and primates, the neurotoxin MPTP induced hypertrophy, as well as hyperplasia of oligodendrocytes in addition to an increase in the total area stained in the nigrostriatum. Thus, oligodendrogliosis was considered to be associated with nigrostriatal degeneration. It was also proposed to reflect early damage to the DA neuron axons and the complex striatal circuits in Parkinsonism. In another study, Takagi et al. (2007) showed that MPTP caused a decrease in CNPase (2′,3′-cyclic nucleotide 3′-phosphodiesterase) protein, thus explaining the toxicity to the striatal oligodendrocytes. Although seemingly contradictory, these findings suggest that the hypertrophied oligodendroglia lose their phenotype, i.e. capacity to synthesize the protein. Our study on human, nigra showed minimal

changes in CNPase with age (Jyothi et al. 2015). Using ex vivo autoradiography, Wu et al. (2010) demonstrated easy entry of [(11)C] MeDAS into the mouse brain as well as preferential and specific labelling of myelinated regions, suggesting its application as a myelin imaging marker.

Protein inclusions present within the glia are termed as glial cell inclusions (GCI). In MSA, GCI containing oligodendroglia expressed higher levels of α-synuclein-ir in the cases of striatonigral degeneration (SND), olivopontocerebellar atrophy (OPCA) and autonomic failure (Dickson et al. 1999; May et al. 2014). The GCIs were related to degeneration of oligodendrocytes surrounding the neurons and blood vessels of the primary and higher cerebral motor cortical areas, 'pyramidal,' 'extra-pyramidal' and cortico-cerebellar systems as well as in the certain autonomic systems (Papp and Lantos 1994; Kato et al. 1991). GCIs were also seen in the hippocampus and pontine nuclei (Horoupian and Dickson 1991), while the olfactory, visual-auditory, somatosensory, cortical association areas and limbic structures were spared. The oligodendroglial degeneration preceded the neuronal damage (Papp and Lantos 1994) and was distinctly different from Lewy bodies (Kato et al. 1991). In autopsied human putamen of MSA patients, neuroinflammation was specifically located within the white matter (Hoffmann et al. 2019).

3.3 Microglia in Basal Ganglia

Lawson et al. (1990) reported that microglial cells are non-uniformly distributed in the major divisions of the brain. More microglia are found in grey matter than white. They are more populous within the basal ganglia, substantia nigra (12%) and hippocampus, than cortex (5%). Morphological typing of microglia suggests that they are sensitive to their microenvironment and transform from the resting or surveillant type to the amoeboid type, i.e. the more pathological type during ageing (Jyothi et al. 2015).

In the neostriatum of embryonic and foetal mice, Sturrock (1981) showed the presence of amoeboid microglia at E13 that phagocytosed cellular remnants, which were absent during adulthood. In the rat brain, at birth, activated microglia migrated into the striatum and attained resting phenotype. Fibronectin appeared to influence this transformation, as round or spindle-shaped brain macrophages reshaped themselves into resting-like morphology during embryogenesis, in vitro. These microglia lacked phagocytic potential and induced oxidative stress through superoxide anion generation. Another extracellular matrix protein laminin blocks the transformation, thus having an antagonistic effect (Chamak and Mallat 1991). LPS pretreatment assisted recovery of dopamine neurons in neonate mice via microglial activation but worsened the same in adult mice. Therefore, activated nigral microglia in development have a neurotrophic potential, while those in the aged are potentially neurotoxic (Sawada et al. 2007).

Injuries or toxins trigger microglial migration and transformation in the basal ganglia. LPS activated caspases 8, 3 and 7 in BV2 microglia-like cells. In the ventral midbrain, the protein kinase C (PKC)-δ-dependent pathway failed to cause

microglial apoptosis, and blocking their overexpression reduced neurotoxicity of LPS (Burguillos et al. 2011). LPS-induced nigrostriatal damage like death of DA neurons and astrocytes in addition to microglial activation was exaggerated by peripheral carrageenan administration, without affecting the BBB (Hernández-Romero et al. 2012). Thus, a mild to moderate peripheral inflammation exacerbates gliosis and DA neuron degeneration caused by another toxic insult, thereby emphasizing the role of a 'second hit' in PD pathogenesis.

Microglia facilitate neuroinflammation following injury, neurotoxic lesions and in the pathogenesis of neurodegenerative disorders. In monkeys, MPTP injection raised a selective microglial response in the substantia nigra, nigrostriatal tract and globus pallidus, but not in striatum. Thus, it was suggested that microglial reactivity may be regionally selective (Hurley et al. 2003). The nigral neurons in Parkinsonian brains overexpress CXCR4 receptors and also its ligand CXCL12. Despite the loss of DA neurons, activated microglial cells were present within the vicinity (Shimoji et al. 2009). In the MPTP-injected mice, upregulation of CXCR4 preceded DA neuron loss. It was initially believed that MPTP caused microglial activation in mouse striatum via overexpression of MAC-1, but not interleukin-1β (Francis et al. 1995). In a disagreement with this study, Nagatsu and Sawada (2005) found that activated microglia overexpressed pro-inflammatory cytokines like IL-6, TNF-α and IL-β, while BDNF was depleted in the CSF and the nigrostriatum of the PD patients as well as MPTP models. These alterations stimulated apoptosis and phagocytosis of DA neurons. Parkinsonian nigra also has amoeboid microglia expressing high levels of NOS, lipocortin-1, COX-1 and COX-2, which were interspersed within neuronal clusters fenced by glial scars (Knott et al. 2000). Vroon et al. (2007) demonstrated that MPTP induced upregulation of striatal IL-1RI mRNA levels. The olfactory bulb rather than the striatum showed relatively higher increase in IL-RII mRNA, underlining the importance of olfactory bulb in PD pathogenesis.

Kurkowska-Jastrzebska et al. (1999) noted elevated expression of MHC class II antigen in the microglia and T cells, but not B cells, in the nigrostriatum of an animal model of PD. CD8+ T cells were common, while CD4+ cells were few. Most lymphocytes were CD44-ir, suggesting an involvement of immune system following neurotoxin administration. Sherer et al. (2003) showed extensive increase in OX-42-immunopositive microglia, in the nigrostriatum of rotenone-injected animals, which preceded DA degeneration. Rotenone caused morphological transformation of microglia from surveillant to phagocytic ones in the nigra, while it was contradicted by their preservation in cortex, thereby suggesting that microglial activation is region specific. Collectively, these studies provide evidence that microglia are major players in neuroinflammation and in clearing the cellular debris/dying/dead cells.

Few studies attempting to control neuroinflammation have received mixed success. Kurkowska-Jastrzebska et al. (1999) found increased expression of ICAM-1 in the microglia and endothelium within the injured regions of their model of PD. Dexamethasone attenuated the neuronal impairment by controlling the glial reaction, inhibiting the infiltration of T cell and expression of MHC class II marker. Takeuchi et al. (1998) reported profuse microglial influx following ethanol injection in rat striatum resulting in necrosis and expression of iNOS, GSA-I-B4, ED-1 and OX-42

that was attenuated by the competitive inhibitor of NOS, i.e. -Ng-nitro-arginine methyl ester (L-NAME). Moon et al. (2009) demonstrated that ghrelin inhibited activation of nigrostriatal microglia and modulates the levels of pro-inflammatory cytokines like TNF-α and IL-1β, as well as the activation of iNOS. Since GHS-R1a is not expressed by microglial cells of SNpc, the metalloproteinase-3 (MMP-3) acts as a potential mediator.

4 Neurotoxins in Understanding Disease Pathogenesis

Neurotoxins have long been considered as classical aids to understand the pathogenic consequences and hence have been used to generate animal models of neurodegeneration. Several studies have effectively used the neurotoxins like ibotenic acid, MPTP, 6-hydroxydopamine, reserpine and rotenone, to understand the pathogenesis of some prominent neurodegenerative disorders. Amongst these, MPTP and 6-hydroxydopamine have explained several aspects of disease pathogenesis (Fig. 1).

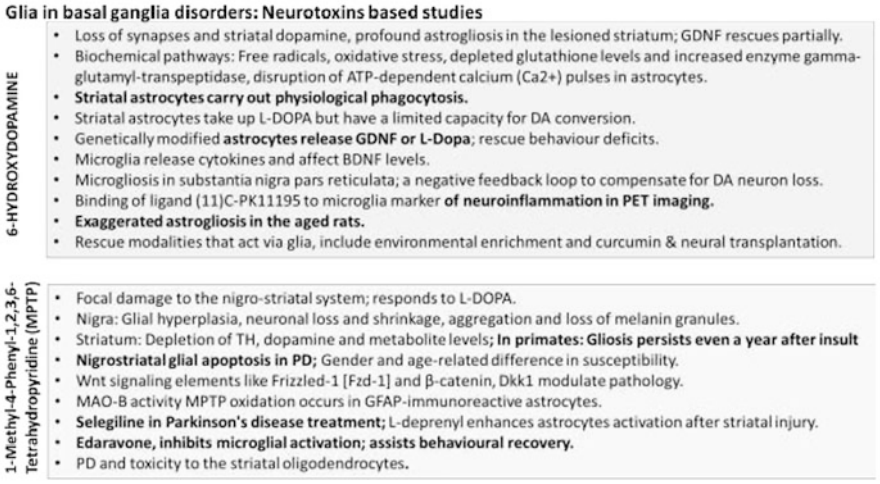

Fig. 1 Neurotoxin based studies elucidating the role of glia in basal ganglia disorders: A major finding of the neurotoxin-based studies has been the occurrence of gliosis (astrogliosis and microgliosis) in the basal ganglia following neurotoxin administration. Other noteworthy findings include the role of glia in phagocytosis and neurotrophy; glial death in late stages of the disease; identification of MAO-B as a risk factor for heightened pathology; Selegiline and Edaravone in PD therapy and identification of ligands for PET-MRI

4.1 Ibotenic Acid-Induced Lesions

Ibotenic acid is an excitatory amino acid that, in long term, has a depressant action on neurons (Curtis et al. 1979). Striatal administration of ibotenic acid at low dose caused widespread astrogliosis and at higher concentration induced neuronal loss (Isacson et al. 1989). Astrogliosis was marked by their morphological hypertrophy, in globus pallidus and SNpr, reminiscing Huntington's disease pathology. It induced fourfold elevation of the GFAP mRNA (Rataboul et al. 1989), and the reactive astrocytes released sulphated glycoprotein-2 or clusterin (Pasinetti and Finch 1991) that is implicated in apoptosis. Danik et al. (1993) showed that clusterin that is normally expressed by neurons is expressed in high amounts by ibotenate-affected reactive astrocytes, resulting in bloating of TH-labelled axons, loss of synapses and formation of vacuoles or dense cytoplasm in the cells and dendrites (Smith et al. 1990). In the due course, astrocytes in the periphery of TH-labelled terminals also show cytoplasmic inclusions. Thus, besides rotenone, ibotenic acid is capable of inducing inclusion formation in animal models of degeneration.

Schauwecker et al. (1998) showed that ibotenate-induced lesions in substantia nigra resulted in rapid although transient increase in the GFAP mRNA in the astrocytes within the striatum, while that in cortex led to a more sustained response along with overexpression of clusterin and ApoE mRNAs. Thus, the upregulation of GFAP was a generalized response of astrocytes, whereas clusterin and ApoE expression were lesion specific that facilitate the carriage of recycled myelin lipids from dying axons to dynamically growing ones.

Pasinetti et al. (1991) showed that cortical ablation with ibotenic acid caused an increase in striatal GFAP concentration, which was matched with a 50% decrease in dopamine and a more dramatic enhancement in its metabolism as well as a reduction in the catalytic activity of TH. Thus, the site of lesion may be important in inducing neurotoxicity. Hanbury et al. (2003) demonstrated that GFAP knockout mice resisted the damage in terms of lesion volume in response to injections of quinolinic acid or 3-nitroproprionic acid within the striatum that result in a metabolic insult, alongside an increase in striatal projection neurons. Besides, the knockout had higher basal levels of GDNF, but not CNTF/NGF. Thus, GFAP expression may be involved in GDNF synthesis to offer partial neuroprotection after a degenerative insult.

4.2 6-Hydroxydopamine-Induced Striatal Damage

A study in the early 1980s evidenced that 6-OHDA induced partial yet sustained reduction of striatal dopamine and loss of synapses (Imamoto et al. 1980). Large-scale necrosis was documented by toluidine blue staining as well as the presence of numerous polysomes and cisternal swelling in the rough ER and Golgi apparatus of neurons, by electron microscopy. The remarkable finding was of accompanying profound astrogliosis in the lesioned striatum (Ogawa et al. 1989). The phenomenon of astrogliosis paved the way further, in the understanding of neurodegenerative

sequelae. Simultaneous expression of laminin in the lesioned area facilitated the migration of reactive astrocytes resulting in degeneration of catecholamine fibres but not striatal neurons. Bowenkamp et al. (1996) reported that administration of 6-OHDA into the medial forebrain bundle affected the nigral neurons, which was partly rescued by GDNF. Unilateral lesions result in enhanced astrogliosis on the ipsilateral side, suggesting that astrogliosis was a consequence of cues from the damaged neurons (Sheng et al. 1993).

Subsequent studies assayed the biochemical outcome of 6-OHDA lesions and thereby the biochemical pathways involved in basal ganglia disorders. It acts via generation of free radicals and oxidative stress, since it depleted glutathione levels and increased enzyme gamma-glutamyl-transpeptidase. Disruption of astrocytic calcium (Ca^{2+}) pulses may characterize neuronal injury, as it may impede Ca^{2+}-dependent trophic functions. Neurotoxins like 6-OHDA and MPP^+ dysregulate the activation of TRPC3. Clearly, this appears to be a novel glial pathway that exacerbates neurotoxic injury (Streifel et al. 2014).

An interesting readout of 6-OHDA studies is the role of astrocytes in phagocytosis. Following 6-OHDA injection, the dopaminergic debris formed free-spheroids/aggregates that stained for a few proteins like TH, DAT and amyloid-precursor protein, but not α-synuclein. The spheroids were LC-3 immunoreactive but LAMP negative, thus suggesting the occurrence of incomplete autophagy and were penetrated by GFAP and S100β immunoreactive astrocyte processes. In an interesting outcome, a few days after the dopaminergic degeneration, the astrocytes stained for TH, DAT and α-synuclein, suggesting that the astrocytes take up intracellular contents of the degenerated cells. Alternatively, this may be indirect evidence that striatal astrocytes perform phagocytosis as a physiological function, to clear debris during the initial stages of PD (Morales et al. 2017). Asanuma et al. (2014) showed that recurrent injections of L-DOPA induced reactive astrocytes on the lesioned side of striatum to express DAT, L-DOPA and DA. Further, in an in vitro model they showed that the withdrawal of extracellular L-DOPA from striatal astrocyte cultures depleted the intracellular L-DOPA level. This suggests that the striatal astrocytes transiently take up L-DOPA, but they have a limited capacity to convert it to DA.

The mode of action of 6-OHDA in rat models of PD is by inducing the microglia to release pro-inflammatory cytokines like TNF-α, IL-6, etc. as well as a reduction in BDNF. This brings to light the possibility that microglia-induced neuroinflammation is the triggering factor for neuronal apoptosis (Nagatsu and Sawada 2005). Another study found enhanced microglial activation more specifically in the pars reticulata (than pars compacta) alongside co-expression of phagocytic markers NG2 proteoglycan and CD68, followed by a reduction in synapsin I- and PSD-95-ir. The activated microglia in the SNpr and GP phagocytosed synaptic elements, like NMDA receptors, which may indicate preferential removal of glutamatergic synapses from the subthalamic nucleus. Thus, microglia may govern the negative feedback loop in the indirect pathway, to offset the loss of DA neurons in PD (Aono et al. 2017). Microglial response in PD was further proven when Cicchetti et al. (2002), using in vivo PET imaging and analyses of autopsied tissues, showed enhanced binding of ligand (11)C-PK11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-

(1-methylpropyl)-3 isoquinoline carboxamide) specifically to microglia in the striatum and SNpc of 6-OHDA lesioned animals and suggested that this ligand could be of use in PET imaging for monitoring neuroinflammation.

Gordon et al. (1997), while inducing unilateral 6-OHDA lesions in the nigrostriatal system, demonstrated that when compared to the young, the aged rat reacted with exaggerated astrogliosis, surplus release of gliotrophic factors and enhanced glial hypersensitivity. However, contrary to this, Pasinetti et al. (1999) suggested that age does not enhance susceptibility of F344 rats to 6-OHDA at the levels of GFAP mRNA. These differences may be due to differences in the species assessed.

Transplantation of cells and foetal brain tissues was proposed to rescue toxin-induced deficits in animal models of PD. Astrocyte transplantation has found a particular mention in studies using 6-OHDA lesion models. Cunningham et al. (1994) co-grafted NGF-releasing astrocytes and adrenal chromaffin cells into the lesioned rat striatum. The chromaffin cells displayed extensive neurite outgrowth and survival and significantly alleviated apomorphine-induced rotational behaviour. Astrocytes genetically modified to release GDNF (Cunningham and Su 2002), or L-DOPA (Lundberg et al. 1996) rescued the amphetamine-induced rotation behaviour in 6-OHDA lesioned animals. In the latter study, L-DOPA-secreting astrocytes survived well, expressed TH and migrated into the surrounding striatum. Thus, astrocytes could be ideal carriers for therapy in PD.

Other glia-mediated rescue modalities include environmental enrichment (Anastasía et al. 2009) and curcumin (Tripanichkul and Jaroensuppaperch 2012), following 6-OHDA-induced toxicity. Thus, the interaction and response of glia in PD have been effectively correlated using the 6-OHDA model.

4.3 MPTP (1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine) Induced PD

Over the last few decades, MPTP has proved to be a valued tool to understand PD pathology. However, earlier studies failed to acknowledge this fact since the magnitude of damage was much lesser than in PD. Jenner and Marsden (1986) found that MPTP administration in primates caused focal damage restricted to the nigrostriatal system that responded to L-DOPA. In a classical study in the MPTP injected marmosets, Gibb (1989) reported changes like glial hyperplasia, neuronal loss and shrinkage, aggregation and loss of melanin granules, nuclear shrinkage, etc. in the substantia nigra. Although the study highlighted glial influence, the deferring factor was the absence of toxicity in the locus coeruleus, substantia innominata and in serotonergic cell groups, which normally harbour widespread degenerative changes in PD. They suggested that MPTP-induced lesions fall short of the classical molecular pathology seen in PD patients. Several studies thereafter provided clinching evidences that intraperitoneal MPTP administration, and some other modalities, induced parkinsonian features in rodent models and that the glia influenced the outcome. A few of them are discussed in the following paragraphs.

Reinhard et al. (1988) suggested that in the substantia nigra, MPTP produced a smaller elevation in GFAP without significantly decreasing dopamine content, while the depletion of dopamine and concomitant increase in GFAP expression, within the striatum, occurred after 7 days. Serra et al. (2002) complemented the findings as MPTP depleted striatal TH and dopamine levels as well as the metabolites in Swiss mice. Simultaneous administration of MPTP and L-α-AA in the nigra accentuated degeneration only when the glial peak was reached, suggesting that the presence of reactive astroglia was critical for the onset of neurotoxicity (Takada et al. 1990).

Muramatsu et al. (2003) confirmed the role of astrocytic calcium, as noted by the upregulation of S-100 in GFAP co-labelled cells during degeneration of DA neurons. They further showed that neuronal death was initiated within 24 h of completion of MPTP regime, and profuse gliosis was noted both in striatum and SNpc, which contradicted the earlier studies (Reinhard et al. 1988). Nigrostriatal glial cell death was seen at a later stage. Thus, proving that glial apoptosis is an important event in PD, which was not observed in other models, including the 6-OHDA models.

O'Callaghan et al. (1990) demonstrated that an acute dose of MPTP elicits strong astrogliotic response in the first instance, which is reduced in response to a second exposure. The first injection causes maximum loss of neurons and a proportionate increase in releasable factors, which may be reduced due to the number of dying neurons in the second instance. Ciesielska et al. (2009) observed that MPTP upregulated GFAP mRNA in the young and ageing males only at early time points, whereas in females it persisted throughout, thus providing explanations for age and sex-related bias in PD.

In vitro studies provide evidence of the role of Wnt1 protein in DAergic neurogenesis. While further exploring their role, L'Episcopo et al. (2013) proved that reactive gliosis and dopaminergic degeneration following MPTP injection were modulated by constituents of Wnt signalling like Frizzled-1 [Fzd-1] and β-catenin. They demonstrated in vitro that activated ventral midbrain astrocytes produced Wnt1. Blocking the signalling with Dkk1 curtailed the astrocyte-induced neuroprotection against MPP^+. Glial involvement in the pathogenesis of PD was further confirmed by Takagi et al. (2007), who showed a significant increase in striatal GFAP and Iba-1 protein levels but a decrease in CNPase (2′,3′-cyclic nucleotide 3′-phosphodiesterase) protein, thus explaining the toxicity to the striatal oligodendrocytes. Thus, persistent gliosis is a signal of irreversible neurodegeneration and is pertinent in designing therapies for the disease. Primate models provide a closer view of PD pathogenesis in humans. Autopsy findings of MPTP-injected monkeys showed that the nigra, but not the striatum, harboured significantly higher congregation of activated astroglia and microglia even a year after administration (Barcia et al. 2004).

Several attempts at identifying therapeutic targets have been designed using MPTP models of PD. Brooks et al. (1989) reported that mice pretreated with fluoxetine were protected against MPTP-induced DA neurotoxicity, but not against MPP^+ ion-induced effects. Later, Youdim and Riederer (1993) provided the seminal evidence that monoamine oxidase-B was inhibited by selegiline, thereby upscaling

polyamines and their N-acetyl derivatives, which highlighted the significance of selegiline in PD treatment. Nakamura et al. (1993) using double immunolabeling showed that monoamine oxidase (MAO) activity was exclusively located to GFAP-ir astrocytes and that it was associated with MPTP oxidation. Thus, MAO-B being a predominantly glial enzyme underlines the importance of glia in metabolizing MPTP. L-Deprenyl is another selective inhibitor of MAO-B. In a study on rat striata, Biagini et al. (1994) demonstrated that L-deprenyl treatment induced significant expression of GFAP around the cannula in the immediate post-lesion period, but not at later time, suggesting that it enhances astrocyte activation during a critical time period following a striatal injury. Revuelta et al. (1997) showed that pretreatment with deprenyl followed by a one-sided transection of the medial forebrain bundle in adult rats induced GFAP-ir in the intact and injured substantia nigra, along with a medio-lateral gradient of decrease. However following axotomy, astrogliosis was seen extensively in striatum. In a later study, Kawasaki et al. (2007) demonstrated that edaravone, a free radical scavenger, inhibited microglial activation and assisted behavioural recovery. Thus, the rescue potential of edaravone rests on blocking the production of reactive oxygen species or peroxynitrites by the microglia. Furuya et al. (2004) showed that knocking out the caspase-11 gene provided resistance against MPTP. Thus, MPTP induces both mitochondrial dysfunction and free radical generation. Collectively, these studies point at the role of glia in pathogenesis as well as treatment of early-stage PD.

Although matrix metalloproteinase-9 (MMP-9) is located within the neurons, MPTP induces STAT upregulation of the protein and mRNA in microglia and astrocytes, alongside a concomitant increase in inflammation. Annese et al. (2015) demonstrated that in chronically injected macaques, persistent inflammation and MMP-9 overexpression were associated with DA neuron loss. Release of MMP-9 by injured neurons favours glia activation; the latter sustain their activated state through autocrine release. Therefore, MMP-9 could be a strategic target to improve outcomes of exaggerated inflammation in PD.

5 Elemental Metals

In a classical study, Lhermitte et al. (1924) proved beyond doubt that iron is augmented in the PD nigra, which was subsequently validated by others using different modalities (Dashtipour et al. 2015). Although required for normal neural functions, iron levels need to be rigorously maintained to avoid oxidative injury. Its presence within oligodendrocytes (Dwork et al. 1988; Gerber and Connor 1989; LeVine and Macklin 1990) and the primary role of oligodendrocytes in iron regulation were long established (Benkovic and Connor 1993). Ferritin and transferrin are responsible for storage and transport of iron, respectively. Iron accumulation in ventral striatum was linked to demyelination and impairments in declarative memory (Steiger et al. 2016) and hence may be of importance in PD with cognitive decline. They showed that in the elderly patients, increase in iron levels was accompanied by

a negative correlation of myelin in the ventral striatum, which predicted individual memory performance.

In experimental models, unilateral injection of kainate or quinolinate into the anterior olfactory nucleus or ventral striatum triggered iron accumulation in the ventral pallidum, globus pallidus and the SNpr. Iron accumulation was noted in glia as well as the neuropil (Shoham et al. 1992). In ageing humans and in monkeys with unilateral MPTP injections, although the iron increased in the nigra, the ferritin levels were normal, indicating that iron accumulation is independent of L-ferritin metabolism (Goto et al. 1996). Dystrophied microglia and astrocytes in aged brains lose their capacity to attenuate metal toxicity and maintain tissue homeostasis, probably due to oxidative stress. In a recent study, Ashraf et al. (2019) showed that basal ganglia are hotspots for age-associated increases in iron; zinc hyper-accumulates in the white matter and copper in the choroid plexus and ventricles. Besides, astrogliosis correlated well with higher levels of iron and zinc.

6 Glia and Growth Factors

Growth factors support the genesis and survival of neurons. Few studies have provided objective evidence of glia as the sources of growth factors; for example, Knott et al. (2002) provided evidence that astrocytes express BDNF under normal conditions. During postnatal development of striatum, the maturation of nNOS-ir interneurons precedes that of parvalbumin-ir interneurons and is modulated by glial BDNF as well as nerve growth factor (NGF) expression. NGF expression also has a regulatory role in glial maturation (Eto et al. 2010). Oo et al. (2005) stated that GDNF mRNA was expressed within medium striatal neurons, but not in glia, in the first postnatal week. The protein was present predominantly in the neuropil within the striosomal patches and in fibres within the major striatal efferent targets, e.g. the globus pallidus, the entopeduncular nucleus, and the SNpr. Tissue extract from DA-depleted striatum has an abundance of trophic activity, which promoted the differentiation of type-1 astrocytes (Shimano et al. 1996). Knott et al. (2002) showed the distribution of BDNF-ir, NT-3-ir as well as the receptors TRK-B and TRK-C in patient and control tissue autopsies. They found BDNF expression in nigral neurons, astrocytes and microglia, in both control and in patient tissues. BDNF expression was absent in caudate-putamen neurons. In patient nigra, more numbers of BDNF-ir ramified glia were found adjacent to the degenerating neurons, along with BDNF-ir in the neuropil. Amoeboid microglia clustered around the neurons in patient nigra. Thus, in disease condition, the glia might infuse neurotrophic support following the feedback from dying or degenerating neurons.

Brito et al. (2004) attempted identification of striatal target cells for BDNF and dopamine, in vitro. BDNF preferentially regulated D (5) receptors in astrocytes and thus regulated their dopamine responsiveness. Besides, developing striatal astrocytes were primary responders to BDNF. Thus, functional interactions between BDNF, dopamine and astrocytes are essential aspects of the differentiating striatal ensemble.

Liver growth factor (LGF) is an albumin-bilirubin complex which protects nigral neurons and supports axonal growth in the striatum, in 6-OHDA-lesioned rats. The DA neuron loss was blocked by treating embryonic midbrain neurons with conditioned medium from LGF-treated glial cultures (GCM-LGF). The neuroprotection was reduced by blocking TNF-α activity. Thus, microglia and TNF-α may protect DA neurons (Gonzalo-Gobernado et al. 2020).

7 Role of Glia in Cell Therapy

In the late 1980s, Zhou and Buchwald (1989) introduced striatal grafts into the striatum of rats using [3H] thymidine autoradiography. They reported that donor astroglia demarcated the transplant from the host tissue. The transplanted striatal cells migrated within the close proximity, whereas the glia migrated further. Within the transplant, donor cells marked by [3H] thymidine stained positively for GABA, substance P and acetylcholinesterase. GABAergic neurons were maximal in the striatal transplant. Astrocytes being an accepted source of neurotrophic factors, Lu et al. (1993) examined the differences between the foetal striatal and cultured astrocyte transplants. They reported that although the grafted astrocytes survived, they were incapable of restoring behavioural deficits, whereas the foetal striatal transplants alleviated the KA-induced rotation behaviour. A human foetal astrocyte cell line (SVG), transfected with cDNA expression vector for TH, could synthesize L-DOPA in the transplanted rat striatum and temporarily rescue the rotational behaviour (Tornatore et al. 1996). However, they were short-lived and attracted CD4 and CD8 expression within the vicinity, as signs of xenograft rejection. Hida et al. (1999) generated Dopa-producing astrocytes in vitro by adenovirus-mediated transduction of human TH gene and transplanted them into hemiparkinsonian rats. The transplanted astrocytes secreted Dopa and reduced methamphetamine-induced rotations for almost 6 weeks. However, over long term, OX-41-positive microglia/macrophages infiltrated the system. Although unsuccessful, this strategy was better than the preceding few studies.

Gopinath et al. (1996) transplanted E14 substantia nigra into the striatum of adult rats and examined them after 2 years. They found alterations in the blood vessels with the presence of macrophages and lymphocytes at the interface. They also found lipofuscin bearing degenerating neurons with fewer intracytoplasmic organelles and also hypertrophied astroglia. These changes represent premature ageing or a slow rejection process. In another study they demonstrated that transplants survive better and without any glial scar in the neonate brain, which is generally rich in growth factors, thus, offering a supportive environment for graft survival (Sable et al. 1997).

Wagner et al. (1999) showed that the overexpression of Nurr1 and factors belonging to the type-1 astrocytes introduced a ventral midbrain-specific DA phenotype in a multipotent neural stem cell line, offering hopes for neuronal replacement in PD. Lipina and Colombo (2001) showed that in MPTP-injected monkeys, bilateral transplantation of astroglia in the striatum assisted partial behavioural recovery

along with an improvement in the clinical motor rating. Thus, these early studies formed the basis of cell therapy in PD.

Foetal cell transplantation studies in humans showed mixed results. Kordower et al. (1997) described the alterations in the autopsied striatum of two patients who had received foetal nigral implants and survived for 18 months. The transplant showed robust survival and occasional HLA-DR staining within the host. However, abundant macrophages as well as T and B cells were noted in some graft sites suggesting that the host immune cells challenge the survival and function of transplants. Mendez et al. (2000) demonstrated that when putaminal transplants obtained from foetal DA cells were a priori exposed to GDNF, both the PD patients showed a twofold increase in putaminal fluorodopa uptake for almost a year. Colombo and Napp (1998) showed that CSF of PD patients treated with L-DOPA had adverse effects on striatum and ventral mesencephalic cells, but not on cerebral cortex cells, in vitro. A prior exposure to foetal astroglial cells eliminated the deleterious effect. Thus, co-transplantation with subcultured astrocytes may facilitate the survival of neurons. Several recent studies have discussed the methods and success of transplants, acquisition of host tissue pathology by the transplanted allografts (Cisbani et al. 2017; Kordower et al. 2017). However, they lack glial angle and hence are not discussed in detail.

8 Alpha-Synuclein

A cluster of neurodegenerative disorders called 'synucleinopathies' are linked to excessive accumulation of α-synuclein. These disorders include PD, dementia with Lewy bodies and MSA. Raghavan et al. (2004) studied α-synuclein expression in foetal, perinatal, paediatric and adolescent brains. In basal ganglia they found somatic expression within the neurons, beginning at 20 week gestation, which persisted through the first few years of life. During late childhood it shifted to the neuropil and persisted there during adulthood. Other entities like endothelial cells, germinal matrix, glia, Purkinje cells, external granular layer, and dentate neurons were not positive. The re-emergence of cytoplasmic localization of α-synuclein in the adult neurons may reflect re-emergence of developmental cues following stress. We noted a similar localization of α-synuclein in ageing human nigra (Alladi et al. 2010b).

The aggregation of γ-synuclein is selectively promoted upon its oxidation at methionine 38 (Met38). Surgucheva et al. (2014) showed co-localization of phospho-α-synuclein and oxidized-γ-synuclein in doughnut-shaped inclusions. Besides, different morphotypes of astrocytes harbour oxidized-γ-synuclein. Sanchez-Guajardo et al. (2013) vaccinated rats with recombinant α-synuclein and noted that overexpression of α-synuclein elevated the anti-α-synuclein antibody titre. In addition, it induced a build-up of CD4 and MHC II-expressing activated microglia in the substantia nigra along with continual incursion of CD4-ir, Foxp3-ir cells panning the nigrostriatum and significantly reduced the striatal aggregates in the vaccinated animals. A persistent increase in striatal-GDNF and presence of IgG

were noted in α-synuclein-overexpressing cells and neurites within the SNpc. These observations highlight the protective attributes of vaccination via stimulation of T cells and activated microglia to bring about immune resilience against α-synuclein.

9 Select Diseases of the Basal Ganglia

9.1 Parkinson's Disease

Another chapter in this compendium is focussed on glia in PD, and hence we have presented it in a limited extent here. Few studies discuss the expanse of astrogliosis in the substantia nigra in PD. Mythri et al. (2011) impressed that in PD, an increase in glutathione-*S*-transferase and astroglial numbers in the non-nigral regions may protect them from oxidative and mitochondrial damage. Further, Tong et al. (2015) demonstrated an inverse relationship between nigral GFAP levels and α-synuclein aggregation, while in MSA it was diametrically opposite; leading to an assumption that α-synuclein might suppress astrogliosis in PD.

Astrocytes constitute the predominant cell type in SNpr and exhibit calcium activities under normal conditions. This activity is both spontaneous and in response to release of neurotransmitters glutamate and GABA. Electrical stimulation of STN disrupts the astrocytic calcium excitability. Thus, SNpr astrocytes sense action in the STN-GPe-SNr loops and may likely provide feedback on the local neuronal activity (Barat et al. 2012). PD is also linked to hyperactivity of SNr neurons. In 6-OHDA-lesioned rats, (Bosson et al. 2015) observed structural plasticity of tripartite glutamatergic synapses and the astrocytic processes in the vicinity of the synapses, suggesting structural and functional reshaping of neuroglial interactions in response to disruption of dopamine transmission.

Carbone et al. (2012) confirmed that riluzole offers neuroprotection via glial proteins in a mammalian model of PD. Although GLT-1 levels remained unaltered, it significantly attenuated GFAP levels on the injected side of the striatum and suppressed reactive astrogliosis. The pars reticulata receives DA from the dendrites of DA neurons in the adjacent pars compacta. Using D1R promoter-controlled mVenus-expressing transgenic mice and double immunolabeling, Nagatomo et al. (2017) showed presence of D1R-ir in the astroglial processes and suggested that the reticulata astrocytes may be a candidate recipient for dendritically released dopamine. However, the physiological role of the various DA receptors in astrocytes is not well understood.

9.2 Multiple System Atrophy (MSA)

MSA is an idiopathic progressive neurological disorder that shows degenerative changes and gliosis in the basal ganglia in addition to regions like brainstem, cerebellum and spinal autonomic nuclei. There is an acknowledged occurrence of glial cytoplasmic inclusions (GCIs) in patient tissues. The symptoms often overlap

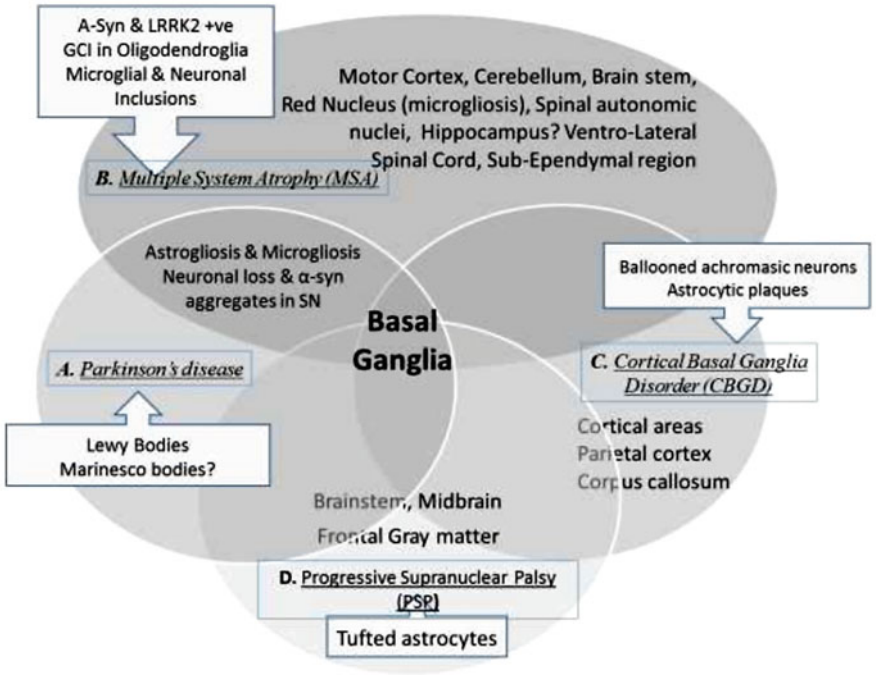

Fig. 2 Schematic illustration of areas involved in pathology of different basal ganglia disorders like (A) Parkinson's disease (PD), (B) MSA, (C) CBGD and (D) PSP. The basal ganglia form the center of the illustration, suggesting its involvement in the pathology of all the four disorders. The additional regions implied are mentioned in each set. PD is marked by Lewy bodies in the substantia nigra

with PD. This may be due to the fact that certain diseases of the basal ganglia present some pathological features in overlapping brain regions (Fig. 2). This results in coinciding manifestations and paired degenerative diseases. Salvesen et al. (2015), while quantifying different cell types, found that MSA patients had substantially fewer neurons in the putamen, globus pallidus and substantia nigra. The caudate nucleus was moderately affected. The oligodendrocytes were much fewer in the putamen and globus pallidus, while astroglia were considerably more. Microgliosis was all pervasive, but maximum microglia were found in the red nucleus that otherwise remained unaffected in the context of neurons, oligodendroglia and astrocytes.

In a landmark study, Papp et al. (1989) demonstrated GCIs by silver staining (hence named argyrophilic), immunocytochemistry and electron microscopy in the patient CNS, with differing intensities to classify them as striatonigral degeneration (SND), olivopontocerebellar atrophy (OPCA) and Shy-Drager syndrome (SDS). The GCIs were localized primarily in the white matter, supplemented by interfascicular oligodendrogliosis and/or loss of myelin staining (Papp and Lantos 1994; Costa and Duyckaerts 1993), indicating that the three syndromes were varied

expressions of MSA. Costa and Duyckaerts (1993) additionally demonstrated the presence of neuronal cytoplasmic inclusions. GCI formation preceded or paralleled neuronal dysfunction in the cerebellar white matter, but their numbers and size were inversely proportional to the severity of the cases of OPCA and were almost absent in the most severe cases (Inoue et al. 1997). The GCIs express microtubule-associated protein MAP5, a foetal antigen, explaining their tubular nature (Arai et al. 1992). The MAP5-glia co-labelled for leucocyte antigen and transferrin, which are microglial and oligodendroglial markers, respectively, suggesting that these two cell types primarily contain the GCIs. May et al. (2014) argued that although the number of mature oligodendrocytes in the corpus callosum was preserved, myelin loss was notable and that α-synuclein delayed the maturation of oligodendrocyte precursor cell via downregulation of myelin basic protein and BDNF. This contradicted the earlier finding of abundant BDNF-positive neurites in the striatum and presence of several BDNF-ir varicose fibres in the GP of patients with severe affliction of the striatum, which was considered to be neuroprotective (Kawamoto et al. 1999). LRRK2, a prominent component of Lewy bodies, was present in the hypertrophied oligodendroglia and co-localized with most α-synuclein-ir GCI and degrading myelin sheaths (Huang et al. 2008), suggesting it to be an early marker. Therefore, neuroinflammation seen in MSA is mostly limited to myeloid cells and closely related to α-synucleinopathy of the oligodendroglia.

Astrocytes and Microglia in MSA

In the early 1980s, Herrick et al. (1983) described complete degeneration of basal ganglia and thalamus, cerebral cortical laminar atrophy, as well as accrual of sudanophil lipid in astrocytes and macrophages of the white matter, in two siblings who died in early infancy. The pathology was suggested to be due to an inherited autosomal recessive disease, representing progressive MSA in utero. Nakamura et al. (2016) described abnormal aggregation of α-synuclein in the subpial astrocytes and those lining the ventricles, in 40% of the patients. The patient brains harboured ramified microglia that expressed Rel A p65in their nucleus, whereas such nuclear location was absent in the striatum (Schwarz et al. 1999). Thus, NF-kappaB/Rel-A complex might mediate microglial activation in MSA. Early progressive activation of microglia in SNpc, overexpression of iNOS and dopaminergic neuronal loss in MSA were subdued by prompt and prolonged minocycline supplementation (Stefanova et al. 2007). Kelly et al. (2018) showed substantial microgliosis in the nigra of OPCA patients, yet it was not linked to the state of activation or expression of cytokines. CX3CL1 precursor protein was depleted, while CX3CR1 protein was increased in MSA. Li et al. (2018) demonstrated that the pbutamen of MSA cases was rich with microglia expressing NLRP3 inflammasome-related and CD-68 proteins and contained phosphorylated α-synuclein-positive GCIs, TH+ve fibre loss, as well as astrogliosis, thus suggesting a correlation between NLRP3 inflammasome and the neurodegeneration. Thus, microglia and astrocytes play critical roles in MSA.

Disease and Symptoms Overlap

Some case reports profess the overlap of disease pathologies in paired neurodegenerative diseases like Parkinson's and MSA (Fig. 3), in view of the prominent evidence of SND and OPCA degeneration, along with the presence of Lewy bodies suggesting the coexistence of two disease processes (Hughes et al. 1991). Gilman et al. (1996) described the presence of GCIs typical of MSA in a case of spinocerebellar ataxia type 1 (SCA1), at autopsy. Nirenberg et al. (2007) described a patient with a late-onset, sporadic and rapidly progressive disorder conforming to MSA-C, which had an abnormal expansion of a SCA3 allele. The presence of α-synuclein-ir GCIs at autopsy confirmed the clinical impression of MSA-C, suggesting that SCA3 abnormality may enhance the risk to develop MSA-C.

Animal Models of MSA

The transgenic mice models of MSA show α-synuclein accumulation and oligodendrogliopathy. Stefanova et al. (2009) demonstrated survival of embryonic-14d striatal allografts assisted by gliosis. Yet the oligodendrocytes that expressed host-specific α-synuclein migrated into the graft reminiscing MSA-like oligodendrogliopathy and neuroinflammation. Fernagut et al. (2014) showed age-related behavioural impairments and brain shrinkage from 12 till 18 months in transgenic mice, alongside ventricular enlargement, cortical atrophy and higher GCI load in the basal ganglia. Bassil et al. (2017) showed that viral-mediated oligodendroglial expression of α-synuclein replicated key features of MSA. Elsewhere, in transgenic MBP29-hα-syn mice that overexpressed human α-synuclein, neuroinflammation was limited to myeloid cells and was proportional to the α-synuclein load in the corpus callosum and striatum at the presymptomatic stage (Hoffmann et al. 2019).

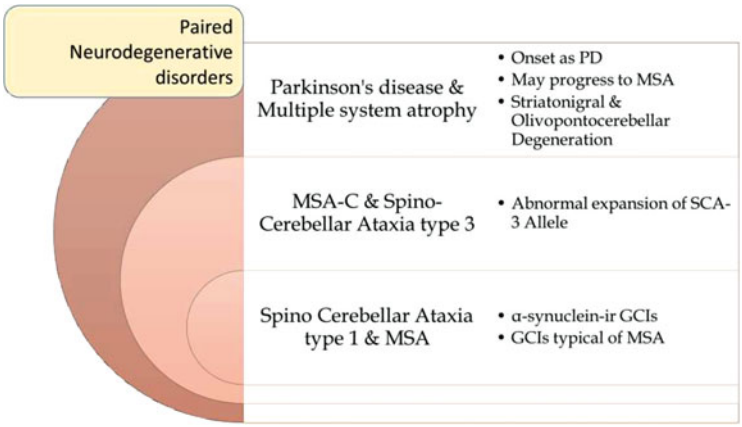

Fig. 3 Basal ganglia disorders that appear to pair either due to overlap of clinical symptoms or pathological signs. MSA often begins as Parkinson's disease and symptoms worsen over a period of time. The cerebellar variant of MSA similarly overlaps with spino-cerebellar ataxia and vice versa

These models have been designed with the expectation that they would be useful in designing or testing therapies. Antidepressant treatments resulted in depletion of α-synuclein-ir cells in the basal ganglia and hippocampus of the MBP1-hα-syn transgenic mice (Valera et al. 2014). The antidepressants modulated the levels of cytokine IL-1β, as confirmed in vitro using astroglial cell line C6. Taken together, these results denote a prompt interaction between α-synuclein-ir GCI oligodendrocytes and neuroinflammation, giving rise to a protective response within the white matter.

9.3 Cortico-Basal Degeneration Vis-à-vis Progressive Supranuclear Palsy

Cortical-basal ganglionic degeneration (CBGD) manifests predominantly as a motor disorder with unique characteristics like focal neuronal loss, swollen achromatic neurons and extensive deposition of abnormal tau in glia and neurons as well as gliosis. Gerhard et al. (2004) using [11C] (R)-PK11195 PET demonstrated microgliosis in cortices and basal ganglia of the patients. In an MRI-based volumetric study, Gröschel et al. (2004) noted atrophied corpus callosum and parietal cortex in CBGD, whereas in PSP a significant decline in volumes of brainstem, midbrain and frontal grey matter was seen.

The pathological findings of CBGD include the presence of 'ballooned neurons' in the cerebral cortex, extensive degeneration in substantia nigra as well as weakly basophilic tau-ir neurofibrillary tangles (NFTs) in substantia nigra, basal ganglia and brainstem (Wakabayashi et al. 1994). As per ultrastructural analysis, NFTs were ubiquitin-ve 15-nm-wide tubules. The cortex and subcortical nuclei housed tau-ir NFT−ve neurons, whereas the tau-ir GCIs were maximally present in the central gyri and brainstem.

Some inclusions were also localized in the oligodendroglia, suggesting a close relationship between CBGD and progressive supranuclear palsy (PSP) (Wakabayashi et al. 1994). Armstrong (2013) on histological sections of white matter of PSP demonstrated high densities of GCI in the basal ganglia, fronto-pontine fibres, cerebellum and superior frontal gyrus. The latter two and GCI were not spatially correlated, indicating significant degeneration of white matter in PSP. Also, vacuolation was linked to loss of neurons in the neighbouring grey matter regions. Damaged axons release abnormal tau which results in GCIs. Gliosis is a response to these alterations.

PSP and CBGD have some shared clinical and pathological properties. Yet both have distinct astrocytic inclusions (Fig. 2). Song et al. (2009) studied the putamen, pons and substantia nigra from Parkinson's disease, MSA, PSP, CBGD and controls to compare astrocyte types and protein expression. MSA, PSP and CBGD had classic reactive astrocytes proportionate to the neurodegeneration and the stage of disease. A sizeable number of subcortical astrocytes specifically accrued α-synuclein in PD and phosphorylated tau in PSP. Thus, tau was associated with CBGD and PSP; hence these diseases belong to the category of tauopathies, whereas

α-synuclein as was prominent in MSA and PD, and therefore they are classified as synucleinopathies. Such heterogeneous astroglial responses in PD, MSA, PSP and CBGD indicate that underlying pathogenic mechanisms differ in each disorder. For example, astrocytic plaques and tufts of abnormal fibres are not found together in CBGD, PSP and atypical PD (Matsumoto et al. 1996). Komori et al. (1998) based on the NINDS neuropathologic criteria suggested that the CBGD corresponded to astrocytic plaques, while tufted astrocytes were typical of PSP. The atypical PSP cases lacked astrocytic inclusions and had novel tau-positive spiny astrocytes.

With reference to the expanse of pathology, the cerebrum is majorly affected in CBGD, while the basal ganglia, diencephalon and brainstem are the affected in PSP. Hattori et al. (2003) described additional areas, e.g. abundance of plaques in caudate nucleus of CBGD brains and tufted astrocytes in thalamus, precentral gyrus, premotor area, red nucleus and superior colliculus in PSP brains. In a large cohort of PSP and CBGD cases, Sato (2014) reported that tufted astrocytes display a centrifugal organization of thin, long, branching aggregated tau protein, whereas astrocytic plaques appear as a corona-like organization of tau aggregates in the distal parts of astrocytic processes. Pathological subtypes of PSP and CBGD were projected to confirm a relationship between clinical phenotypes, pathological distribution and degenerative changes. For instance, PSP cases are subdivided into typical PSP, CBGD-like and pallido-nigro-luysian type. CBGD is divided into three pathological subtypes: typical CBGD type, basal ganglia-predominant type and PSP-like. Yet each case appears as a challenge to the neurologist.

9.4 Wilson's Disease

The copper metabolism disorder, also known as hepatolenticular degeneration or Wilson's disease (WD), was first defined in 1912. It presents in infancy and as well as in young adults (Bandmann et al. 2015; Aggarwal and Bhatt 2018). It is an autosomal recessive disorder that manifests because of a defective copper-transporting ATPase ATP7B gene, which is essential for secretion of biliary copper and loading of copper onto ceruloplasmin (Scheiber et al. 2017). It results in compromised excretion of copper in liver and its accumulation in some tissues (Capone and Azzam 2018). The pathophysiology emerges from the inability of the biliary system to excrete copper, resulting in its gradual accumulation, initiated in the liver that ends in the brain (Pfeiffer 2011). Toxic copper depositions cause multiple anomalies, viz. oxidative stress, direct inhibition of proteins and impaired mitochondrial function, leading to symptoms related to the liver, kidneys, musculoskeletal system as well as neuropsychiatric symptoms (Scheiber et al. 2017). In copper-administered rats, tigrolysis of thalamic nerve cells was a prominent feature. Besides, copper depositions were observed in thalamic glia cells and Descemet's membrane of the cornea in addition to other cell types (Narasaki 1980).

Striatal lesions are the most distinguishable ones in the brains of WD patients with neurological symptoms (Wilson 1912). These can be more diffuse, including in the regions like pons, midbrain, thalamus, dentate nucleus and occasionally in corpus callosum as well as cortex. Occasional cases describe widespread cortical-subcortical lesions (Poujois et al. 2017). The numbers and types of cells appear to increase due to the proliferation of Alzheimer types of glia (modified astrocytes) and Opalski cells. Surprisingly, the copper content in the cortex does not correlate with the severity of the neuropathology or neuropsychiatric symptoms (Poujois et al. 2017).

The microglial ultrastructure showed major adaptation of intermediate forms, i.e. between rod, ramified and amoeboid microglia (Lewandowska et al. 2004). Demyelinating type of WD was observed at autopsy in a 17-year-old male, with the presence of extensive myelin loss and tissue sponginess in the white matter of the cortex and cerebellum. Similarly, autopsied brains showed scattered Alzheimer type I, II and Opalski glia in basal ganglia and cortex as well as presence of copper granules in the cytoplasm of liver parenchymal cells. This was accompanied by spongy degeneration leading to cavity formation with insufficient glial fibre proliferation. The hippocampal formation, calcarine areas, amygdaloid nuclei and the hypothalamus were relatively better preserved (Miyakawa and Murayama 1976; Shimoji et al. 1987). Surprisingly, an immunohistochemical study of autopsied brain revealed copper accumulation within Olig-2 ir cells (Nishimuta et al. 2018). In two cases, severe white matter lesions of the superior gyri with profound neuronal loss and capillary proliferation were reported. Alzheimer's type I (AlzgI/A-I) and II (AlzgII/A-II) astrocytes and numerous Opalski cells showed unbound copper deposition in the white matter suggesting neurotoxic effect of copper (Mikol et al. 2005).

In an MRI-based study, hyperintensity in the basal ganglia was linked to iron rather than copper deposition (Dusek et al. 2018). The distribution patterns of A-I and A-II astrogliosis were distinct: A-I cells were predominantly present in the demyelinating areas with profound reactive astrogliosis, while the A-II cells were scattered in both the grey and white matter, in which both the flat and process-bearing astrocytes were affected (Ma et al. 1988). The animal models described recently include, i.e. Long-Evans Cinnamon rat, the toxic-milk mouse, ATP7B knockout mouse and the Labrador Retriever that mimic human hepatic WD, resulting in improved understanding of the disease, although they lack the neurological phenotype (Reed et al. 2018). Gene therapy using different AAV (adeno-associated vector) systems provides long-term correction of copper metabolism in animals, which can also be extrapolated to humans (Murillo et al. 2019).

9.5 Other Basal Ganglia Disorders

Amongst the earliest studies, Friede (1979) provided brightfield and electron microscopy-based morphological evidence of abnormal pigmentation within the astrocytes of the striatum, pallidum and substantia nigra of two unrelated patients with no evidence of any neurological disorder observations, indicating that astrocyte

pathology precedes neuronal one. Bronson and Schoene (1980) mentioned the occurrence of iron pigment and spheroid-like structures in the nigral and pallidal glia of Old World monkeys, maintained in captivity. These eosinophilic as well as argyrophilic inclusions were periodic acid-Schiff, iron and Luxol fast blue negative and lacked normal organelles. They equated their findings with spheroid degeneration diseases like Hallervorden-Spatz disease.

In a patient with juvenile-onset generalized dystonia, Gibb et al. (1992) described preserved pockets in between islands of excessive gliosis as well as cell loss, within the caudate nucleus and putamen. While the dorsal aspects showed confluent gliosis, the ventral parts were spared. The pathological findings mimicked those reported in cranial dystonia. Miyamoto et al. (2001) presented a case of frontotemporal dementia and early-onset Parkinsonism with PSP-like features. The neuronal loss was much higher in the temporal and frontal cortex, dentate nucleus, globus pallidus, SNpc and red nucleus. Changes in the fibrils were noted in tau immunostained neurons and glia. Dainese et al. (2013) showed polyglucosan bodies in basal ganglia as well as in several peripheral tissues, including the diaphragm, peripheral nerves and cerebral white matter, located exclusively in astrocytes. Since brain glycogen is also exclusively metabolized in astrocytes, this observation sheds light on the pathophysiology of adult polyglucosan body disease. Machado-Joseph disease (MJD) is a neurodegenerative disorder that is related to abnormal CAG expansion, resulting in an expanded polyglutamine tract in ataxin-3. Gonçalves et al. (2013) used lentiviral vectors encoding mutant ataxin-3, to transduce the adult mice striatum and mimic MJD. They found that synaptotoxicity and gliosis were forerunners, as they preceded neuronal death. Secondly, caffeine and A2A-R deletion reduced the striatal pathology and proposed A2A-Rs as a novel candidate for therapy in MJD.

Neuronal degeneration in basal ganglia, amygdala and thalamus was prominent in dementia in PDD, and hence it was thought to be of subcortical origin. In PD + AD the subcortical degeneration was compounded by effects on the cortical neurons and long projection fibres coursing through cerebral white matter. Thus, both the dementias were considered to be distinct in origin (de la Monte et al. 1989a, b). Khundakar et al. (2011) found moderate reductions in neuronal density in the caudate nucleus, more specifically in the dorsolateral and ventromedial aspects, of the depressed group. However, glial density and neuronal volume were unaltered in subgroups of early and late-onset depression.

Thus, literature review of past few decades reveals that in almost all basal ganglia disorders, glial pathology takes reasonable prominence. It mostly precedes and invariably parallels the neuronal dysfunction that ultimately results in disease manifestation. In an exciting step forward, recent studies have focussed on detecting neuroinflammation at an early stage using radio-imaging modalities and correlating them with peripheral markers, for instance, in dementia with Lewy bodies (Surendranathan et al. 2018). Secondly, it might be useful to establish a correlation between the glial types involved in each disease or the sequence of their involvement. Identification of glial cell types that dominate or those that are affected in specific diseases would provide better cues for therapy. The critical denomination for future studies would be to identify the signalling cascades in glial pathology that

may help identify predominance and sequence of activation of specific proteins. The ultimate goal would be to determine whether glial cells are the victims or the godfathers of neurodegeneration.

Acknowledgement The authors gratefully acknowledge the financial support of DBT (grant no. BT/PR12578/MED/30/1462/2014) to PAA. BB is a NIMHANS Fellow.

Conflicts of Interest None.

References

Aggarwal A, Bhatt M (2018) Advances in treatment of Wilson disease. Tremor Other Hyperkinet Mov 8:525. https://doi.org/10.7916/D841881D

Alladi PA, Mahadevan A, Yasha TC, Raju TR, Shankar SK, Muthane U (2009) Absence of age-related changes in nigral dopaminergic neurons of Asian Indians: relevance to lower incidence of Parkinson's disease. Neuroscience 159:236–245. https://doi.org/10.1016/j.neuroscience.2008.11.051

Alladi PA, Mahadevan A, Shankar SK, Raju TR, Muthane U (2010a) Expression of GDNF receptors GFRα1 and RET is preserved in substantia nigra pars compacta of aging Asian Indians. J Chem Neuroanat 40:43–52. https://doi.org/10.1016/j.jchemneu.2010.03.007

Alladi PA, Mahadevan A, Vijayalakshmi K, Muthane U, Shankar SK, Raju TR (2010b) Ageing enhances α-synuclein, ubiquitin and endoplasmic reticular stress protein expression in the nigral neurons of Asian Indians. Neurochem Int 57:530–539. https://doi.org/10.1016/j.neuint.2010.06.018

Anastasía A, Torre L, Erausquin GAD, Mascó DH (2009) Enriched environment protects the nigrostriatal dopaminergic system and induces astroglial reaction in the 6-OHDA rat model of Parkinson's disease. J Neurochem 109:755–765. https://doi.org/10.1111/j.1471-4159.2009.06001.x

Annese V, Barcia C, Ros-Bernal F, Gómez A, Ros CM, De Pablos V, Fernández-Villalba E, De Stefano ME, Herrero M-T (2013) Evidence of oligodendrogliosis in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism. Neuropathol Appl Neurobiol 39:132–143. https://doi.org/10.1111/j.1365-2990.2012.01271.x

Annese V, Herrero M-T, Di Pentima M, Gomez A, Lombardi L, Ros CM, De Pablos V, Fernandez-Villalba E, De Stefano ME (2015) Metalloproteinase-9 contributes to inflammatory glia activation and nigro-striatal pathway degeneration in both mouse and monkey models of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism. Brain Struct Funct 20:703–727. https://doi.org/10.1007/s00429-014-0718-8

Aono H, Choudhury ME, Higaki H, Miyanishi K, Kigami Y, Fujita K, Akiyama J, Takahashi H, Yano H, Kubo M, Nishikawa N, Nomoto M, Tanaka J (2017) Microglia may compensate for dopaminergic neuron loss in experimental Parkinsonism through selective elimination of glutamatergic synapses from the subthalamic nucleus. Glia 65:1833–1847. https://doi.org/10.1002/glia.23199

Arai N, Nishimura M, Oda M, Morimatsu Y, Ohe R, Nagatomo H (1992) Immunohistochemical expression of microtubule-associated protein 5 (MAP5) in glial cells in multiple system atrophy. J Neurol Sci 109:102–106. https://doi.org/10.1016/0022-510X(92)90101-P

Armstrong RA (2013) White matter pathology in progressive supranuclear palsy (PSP): a quantitative study of 8 cases. Clin Neuropathol 32:399–405. https://doi.org/10.5414/NP300608

Asanuma M, Miyazaki I, Murakami S, Diaz-Corrales FJ, Ogawa N (2014) Striatal astrocytes act as a reservoir for L-DOPA. PLoS One 9:e106362. https://doi.org/10.1371/journal.pone.0106362

Ashraf A, Michaelides C, Walker TA, Ekonomou A, Suessmilch M, Sriskanthanathan A, Abraha S, Parkes A, Parkes HG, Geraki K, So P-W (2019) Regional distributions of iron, copper and zinc and their relationships with glia in a normal aging mouse model. Front Aging Neurosci 11:351. https://doi.org/10.3389/fnagi.2019.00351

Bal A, Bachelot T, Savasta M, Manier M, Verna JM, Benabid AL, Feuerstein C (1994) Evidence for dopamine D2 receptor mRNA expression by striatal astrocytes in culture: in situ hybridization and polymerase chain reaction studies. Mol Brain Res 23:204–212. https://doi.org/10.1016/0169-328X(94)90227-5

Bandmann O, Weiss KH, Kaler SG (2015) Wilson's disease and other neurological copper disorders. Lancet Neurol 14:103–113. https://doi.org/10.1016/S1474-4422(14)70190-5

Barat E, Boisseau S, Bouyssières C, Appaix F, Savasta M, Albrieux M (2012) Subthalamic nucleus electrical stimulation modulates calcium activity of nigral astrocytes. PLoS One 7:e41793. https://doi.org/10.1371/journal.pone.0041793

Barcia C, Bahillo AS, Fernández-Villalba E, Bautista V, Poza MPY, Fernández-Barreiro A, Hirsch EC, Herrero M-T (2004) Evidence of active microglia in substantia nigra pars compacta of parkinsonian monkeys 1 year after MPTP exposure. Glia 46:402–409. https://doi.org/10.1002/glia.20015

Bassil F, Guerin PA, Dutheil N, Li Q, Klugmann M, Meissner WG, Bezard E, Fernagut P-O (2017) Viral-mediated oligodendroglial alpha-synuclein expression models multiple system atrophy. Mov Disord 32:1230–1239. https://doi.org/10.1002/mds.27041

Benkovic SA, Connor JR (1993) Ferritin, transferrin, and iron in selected regions of the adult and aged rat brain. J Comp Neurol 338(1):97–113

Beyer C, Pilgrim C, Reisert I, Misgeld U (1991) Cells from embryonic rat striatum cocultured with mesencephalic glia express dopaminergic phenotypes. Neurosci Lett 128(1):1–3

Biagini G, Frasoldati A, Fuxe K, Agnati LF (1994) The concept of astrocyte-kinetic drug in the treatment of neurodegenerative diseases: evidence for l-deprenyl-induced activation of reactive astrocytes. Neurochem Int 25:17–22. https://doi.org/10.1016/0197-0186(94)90047-7

Birling M-C, Price J (1998) A study of the potential of the embryonic rat telencephalon to generate oligodendrocytes. Dev Biol 193:100–113. https://doi.org/10.1006/dbio.1997.8763

Bosson A, Boisseau S, Buisson A, Savasta M, Albrieux M (2015) Disruption of dopaminergic transmission remodels tripartite synapse morphology and astrocytic calcium activity within substantia nigra pars reticulata. Glia 63:673–683. https://doi.org/10.1002/glia.22777

Bottcher J (1975) Morphology of the basal ganglia in Parkinson's disease. Acta Neurol Scand Suppl 62:1–87

Bøttcher J (1978) Corpus striatum in infants under the age of one year: a quantitative morphological study. J Neurol Sci 38:163–169. https://doi.org/10.1016/0022-510X(78)90062-X

Bowenkamp KE, David D, Lapchak PL, Henry MA, Granholm A-C, Hoffer BJ, Mahalik TJ (1996) 6-Hydroxydopamine induces the loss of the dopaminergic phenotype in substantia nigra neurons of the rat. Exp Brain Res 111:1–7. https://doi.org/10.1007/BF00229549

Brito V, Beyer C, Küppers E (2004) BDNF-dependent stimulation of dopamine D5 receptor expression in developing striatal astrocytes involves PI3-kinase signaling. Glia 46(3):284–295

Bronson RT, Schoene WC (1980) Spontaneous pallido-nigral accumulation of iron pigment and spheroid-like structures in macaque monkeys. J Neuropathol Exp Neurol 39:181–196. https://doi.org/10.1097/00005072-198003000-00007

Brooks WJ, Jarvis MF, Wagner GC (1989) Astrocytes as a primary locus for the conversion MPTP into MPP+. J Neural Transm 76:1–12. https://doi.org/10.1007/BF01244987

Burguillos MA, Hajji N, Englund E, Persson A, Cenci AM, Machado A, Cano J, Joseph B, Venero JL (2011) Apoptosis-inducing factor mediates dopaminergic cell death in response to LPS-induced inflammatory stimulus: evidence in Parkinson's disease patients. Neurobiol Dis 41:177–188. https://doi.org/10.1016/j.nbd.2010.09.005

Capone K, Azzam RK (2018) Wilson's disease: a review for the general pediatrician. Pediatr Ann 47:e440–e444. https://doi.org/10.3928/19382359-20181026-01

Carbone M, Duty S, Rattray M (2012) Riluzole neuroprotection in a Parkinson's disease model involves suppression of reactive astrocytosis but not GLT-1 regulation. BMC Neurosci 13:38. https://doi.org/10.1186/1471-2202-13-38

Chamak B, Mallat M (1991) Fibronectin and laminin regulate the in vitro differentiation of microglial cells. Neuroscience 45:513–527. https://doi.org/10.1016/0306-4522(91)90267-R

Cicchetti F, Brownell AL, Williams K, Chen YI, Livni E, Isacson O (2002) Neuroinflammation of the nigrostriatal pathway during progressive 6 – OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. Eur J Neurosci 15(6):991–998

Ciesielska A, Joniec I, Kurkowska-Jastrzębska I, Cudna A, Przybyłkowski A, Członkowska A, Członkowski A (2009) The impact of age and gender on the striatal astrocytes activation in murine model of Parkinson's disease. Inflamm Res 58:747–753. https://doi.org/10.1007/s00011-009-0026-6

Cisbani G, Maxan A, Kordower JH, Planel E, Freeman TB, Cicchetti F (2017) Presence of tau pathology within foetal neural allografts in patients with Huntington's and Parkinson's disease. Brain 140:2982–2992. https://doi.org/10.1093/brain/awx255

Colombo JA, Napp MI (1998) Cerebrospinal fluid from l-Dopa-treated Parkinson's disease patients is dystrophic for various neural cell typesex vivo: effects of astroglia. Exp Neurol 154:452–463. https://doi.org/10.1006/exnr.1998.6910

Costa C, Duyckaerts C (1993) Oligodendroglial and neuronal inclusions in multiple system atrophy. Curr Opin Neurol 6(6):865–871

Cunningham LA, Su C (2002) Astrocyte delivery of glial cell line-derived neurotrophic factor in a mouse model of Parkinson's disease. Exp Neurol 174:230–242. https://doi.org/10.1006/exnr.2002.7877

Cunningham LA, Short MP, Breakefield XO, Bohn MC (1994) Nerve growth factor released by transgenic astrocytes enhances the function of adrenal chromaffin cell grafts in a rat model of Parkinson's disease. Brain Res 658:219–231. https://doi.org/10.1016/S0006-8993(09)90029-4

Curtis DR, Lodge D, McLennan H (1979) The excitation and depression of spinal neurones by ibotenic acid. J Physiol 291:19–28. https://doi.org/10.1113/jphysiol.1979.sp012796

Dahl D (1981) The vimentin-GFA protein transition in rat neuroglia cytoskeleton occurs at the time of myelination. J Neurosci Res 6:741–748. https://doi.org/10.1002/jnr.490060608

Dainese L, Monin M-L, Demeret S, Brochier G, Froissart R, Spraul A, Schiffmann R, Seilhean D, Mochel F (2013) Abnormal glycogen in astrocytes is sufficient to cause adult polyglucosan body disease. Gene 515:376–379. https://doi.org/10.1016/j.gene.2012.12.065

Danik M, Chabot J-G, Hassan-gonzalez D, Suh M, Quirion R (1993) Localization of sulfated glycoprotein-2/clusterin mRNA in the rat brain by in situ hybridization. J Comp Neurol 334:209–227. https://doi.org/10.1002/cne.903340205

Dashtipour K, Liu M, Kani C, Dalaie P, Obenaus A, Simmons D, Gatto NM, Zarifi M (2015) Iron accumulation is not homogenous among patients with Parkinson's disease. Parkinsons Dis 2015:324843. https://doi.org/10.1155/2015/324843

de la Monte SM, Hutchins GM, Moore GW (1989a) Racial differences in the etiology of dementia and frequency of Alzheimer lesions in the brain. J Natl Med Assoc 81:644–652

de la Monte SM, Wells SE, Hedley-Whyte ET, Growdon JH (1989b) Neuropathological distinction between Parkinson's dementia and Parkinson's plus Alzheimer's disease. Ann Neurol 26:309–320. https://doi.org/10.1002/ana.410260302

Denis-Donini S, Estenoz M (1988) Interneurons versus efferent neurons: heterogeneity in their neurite outgrowth response to glia from several brain regions. Dev Biol 130:237–249. https://doi.org/10.1016/0012-1606(88)90430-7

Denis-Donini S, Glowinski J, Prochiantz A (1983) Specific influence of striatal target neurons on the in vitro outgrowth of mesencephalic dopaminergic neurites: a morphological quantitative study. J Neurosci 3:2292–2299. https://doi.org/10.1523/JNEUROSCI.03-11-02292.1983

Denis-Donini S, Glowinski J, Prochiantz A (1984) Glial heterogeneity may define the three-dimensional shape of mouse mesencephalic dopaminergic neurones. Nature 307(5952):641–643

di Porzio U, Daguet M-C, Glowinski J, Prochiantz A (1980) Effect of striatal cells on in vitro maturation of mesencephalic dopaminergic neurones grown in serum-free conditions. Nature 288:370–373. https://doi.org/10.1038/288370a0

Dickson DW, Lin W, Liu W-K, Yen S-H (1999) Multiple system atrophy: a sporadic synucleinopathy. Brain Pathol 9:721–732. https://doi.org/10.1111/j.1750-3639.1999.tb00553.x

Domaradzka-Pytel B, Ludkiewicz B, Jagalska-Majewska H, Moryś J (2000) Immunohistochemical study of microglial and astroglial cells during postnatal development of rat striatum. Folia Morphol (Warsz) 58(4):315–323

Dong JF, Detta A, Bakker MHM, Hitchcock ER (1993) Direct interaction with target-derived glia enhances survival but not differentiation of human fetal mesencephalic dopaminergic neurons. Neuroscience 56:53–60. https://doi.org/10.1016/0306-4522(93)90561-S

Drukarch B, Schepens E, Stoof JC, Langeveld CH, Van Muiswinkel FL (1998) Astrocyte-enhanced neuronal survival is mediated by scavenging of extracellular reactive oxygen species. Free Radic Biol Med 25:217–220. https://doi.org/10.1016/S0891-5849(98)00050-1

Dusek P, Skoloudik D, Maskova J, Huelnhagen T, Bruha R, Zahorakova D, Niendorf T, Ruzicka E, Schneider SA, Wuerfel J (2018) Brain iron accumulation in Wilson's disease: a longitudinal imaging case study during anticopper treatment using 7.0 T MRI and transcranial sonography. J Magn Reson Imaging 47(1):282–285

Dwork AJ, Schon EA, Herbert J (1988) Nonidentical distribution of transferrin and ferric iron in human brain. Neuroscience 27:333–345. https://doi.org/10.1016/0306-4522(88)90242-4

Eto R, Abe M, Kimoto H, Imaoka E, Kato H, Kasahara J, Araki T (2010) Alterations of interneurons in the striatum and frontal cortex of mice during postnatal development. Int J Dev Neurosci 28:359–370. https://doi.org/10.1016/j.ijdevneu.2010.04.004

Fernagut PO, Meissner WG, Biran M, Fantin M, Bassil F, Franconi JM, Tison F (2014) Age-related motor dysfunction and neuropathology in a transgenic mouse model of multiple system atrophy. Synapse 68:98–106. https://doi.org/10.1002/syn.21719

Francis JW, Von Visger J, Markelonis GJ, Oh TH (1995) Neuroglial responses to the dopaminergic neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mouse striatum. Neurotoxicol Teratol 17:7–12. https://doi.org/10.1016/0892-0362(94)00048-I

Friede RL (1979) Striato-nigral astrocytic melanization. J Neurol 220:149–156. https://doi.org/10.1007/BF00705533

Furuya T, Hayakawa H, Yamada M, Yoshimi K, Hisahara S, Miura M, Mizuno Y, Mochizuki H (2004) Caspase-11 mediates inflammatory dopaminergic cell death in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. J Neurosci 24:1865–1872. https://doi.org/10.1523/JNEUROSCI.3309-03.2004

Gerber MR, Connor JR (1989) Do oligodendrocytes mediate iron regulation in the human brain? Ann Neurol 26:95–98. https://doi.org/10.1002/ana.410260115

Gerhard A, Watts J, Trender-Gerhard I, Turkheimer F, Banati RB, Bhatia K, Brooks DJ (2004) In vivo imaging of microglial activation with [11C](R)-PK11195 PET in corticobasal degeneration. Mov Disord 19:1221–1226. https://doi.org/10.1002/mds.20162

Gibb WRG (1989) The pathology of parkinsonian disorders. In: Quinn NP, Jenner PG (eds) Disorders of movement: clinical, pharmacological and physiological aspects. Academic, London, pp 33–57

Gibb WRG, Kilford L, Marsden CD (1992) Severe generalised dystonia associated with a mosaic pattern of striatal gliosis. Mov Disord 7(3):217–223

Gilman S, Sima AAF, Junck L, Kluin KJ, Koeppe RA, Lohman ME, Little R (1996) Spinocerebellar ataxia type 1 with multiple system degeneration and glial cytoplasmic inclusions. Ann Neurol 39:241–255. https://doi.org/10.1002/ana.410390214

Gonçalves N, Simões AT, Cunha RA, de Almeida LP (2013) Caffeine and adenosine A2A receptor inactivation decrease striatal neuropathology in a lentiviral – based model of Machado–Joseph disease. Ann Neurol 73(5):655–666

Gonzalo-Gobernado R, Reimers D, Casarejos MJ, Calatrava Ferreras L, Vallejo-Muñoz M, Jiménez-Escrig A, Diaz-Gil JJ, Ulzurrun de Asanza GM, Bazán E (2020) Liver growth factor induces glia-associated neuroprotection in an in vitro model of Parkinson's disease. Brain Sci 10(5):315

Gopinath G, Sailaja K, Tandon PN (1996) Long-term nigral transplants in rat striatum: an electron microscopic study. Int J Dev Neurosci 14:453–460. https://doi.org/10.1016/0736-5748(96)00003-2

Gordon MN, Schreier WA, Ou X, Holcomb LA, Morgan DG (1997) Exaggerated astrocyte reactivity after nigrostriatal deafferentation in the aged rat. J Comp Neurol 388(1):106–119

Goto K, Mochizuki H, Imai H, Akiyama H, Mizuno Y (1996) An immuno-histochemical study of ferritin in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced hemiparkinsonian monkeys. Brain Res 724:125–128. https://doi.org/10.1016/0006-8993(96)00284-3

Graybiel AM, Ragsdale CW (1978) Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci U S A 75:5723–5726. https://doi.org/10.1073/pnas.75.11.5723

Gröschel K, Hauser T-K, Luft A, Patronas N, Dichgans J, Litvan I, Schulz JB (2004) Magnetic resonance imaging-based volumetry differentiates progressive supranuclear palsy from corticobasal degeneration. NeuroImage 21:714–724. https://doi.org/10.1016/j.neuroimage.2003.09.070

Halliday AL, Cepko CL (1992) Generation and migration of cells in the developing striatum. Neuron 9:15–26. https://doi.org/10.1016/0896-6273(92)90216-Z

Hanbury R, Ling ZD, Wuu J, Kordower JH (2003) GFAP knockout mice have increased levels of GDNF that protect striatal neurons from metabolic and excitotoxic insults. J Comp Neurol 461:307–316. https://doi.org/10.1002/cne.10667

Hansson E (1986) Primary cultures from defined brain areas. III. Effects of seeding time on [3H]l-glutamate transport and glutamine synthetase activity. Dev Brain Res 24:203–209. https://doi.org/10.1016/0165-3806(86)90188-4

Hansson E, Rönnbäck L (1988) Neurons from substantia nigra increase the efficacy and potency of second messenger arising from striatal astroglia dopamine receptor. Glia 1:393–397. https://doi.org/10.1002/glia.440010606

Hattori M, Okuno Y, Goto S, Kanehisa M (2003) Development of a chemical structure comparison method for integrated analysis of chemical and genomic information in the metabolic pathways. J Am Chem Soc 125(39):11853–11865

Hernández-Romero MC, Delgado-Cortés MJ, Sarmiento M, de Pablos RM, Espinosa-Oliva AM, Argüelles S, Bández MJ, Villarán RF, Mauriño R, Santiago M, Venero JL, Herrera AJ, Cano J, Machado A (2012) Peripheral inflammation increases the deleterious effect of CNS inflammation on the nigrostriatal dopaminergic system. Neurotoxicology 33:347–360. https://doi.org/10.1016/j.neuro.2012.01.018

Herrick MK, Strefling AM, Urich H (1983) Intrauterine multisystem atrophy in siblings: a new genetic syndrome? Acta Neuropathol (Berl.) 61:65–70. https://doi.org/10.1007/BF00688388

Hida H, Hashimoto M, Fujimoto I, Nakajima K, Shimano Y, Nagatsu T, Mikoshiba K, Nishino H (1999) Dopa-producing astrocytes generated by adenoviral transduction of human tyrosine hydroxylase gene: in vitro study and transplantation to hemiparkinsonian model rats. Neurosci Res 35:101–112. https://doi.org/10.1016/S0168-0102(99)00073-5

Hoffmann A, Ettle B, Battis K, Reiprich S, Schlachetzki JCM, Masliah E, Wegner M, Kuhlmann T, Riemenschneider MJ, Winkler J (2019) Oligodendroglial α-synucleinopathy-driven neuroinflammation in multiple system atrophy. Brain Pathol 29:380–396. https://doi.org/10.1111/bpa.12678

Horoupian DS, Dickson DW (1991) Striatonigral degeneration, olivopontocerebellar atrophy and "atypical" Pick disease. Acta Neuropathol (Berl.) 81:287–295. https://doi.org/10.1007/BF00305870

Howard S, Landry C, Fisher R, Bezouglaia O, Handley V, Campagnoni A (1998) Postnatal localization and morphogenesis of cells expressing the dopaminergic D2 receptor gene in rat brain: expression in non-neuronal cells. J Comp Neurol 391:87–98. https://doi.org/10.1002/(SICI)1096-9861(19980202)391:1<87::AID-CNE8>3.0.CO;2-N

Huang Y, Song YJC, Murphy K, Holton JL, Lashley T, Revesz T, Gai W-P, Halliday GM (2008) LRRK2 and parkin immunoreactivity in multiple system atrophy inclusions. Acta Neuropathol (Berl.) 116:639–646. https://doi.org/10.1007/s00401-008-0446-3

Hughes AJ, Daniel SE, Lees AJ (1991) Idiopathic Parkinson's disease combined with multiple system atrophy. A clinicopathological report. Mov Disord 6:342–346. https://doi.org/10.1002/mds.870060413

Hurley SD, O'Banion MK, Song DD, Arana FS, Olschowka JA, Haber SN (2003) Microglial response is poorly correlated with neurodegeneration following chronic, low-dose MPTP administration in monkeys. Exp Neurol 184:659–668. https://doi.org/10.1016/S0014-4886(03)00273-5

Imamoto K, Nagai T, Kimura H, Maeda T (1980) Effect of neonatal administrations of 6-OHDA on the brain development I. Alteration in the striatum. Arch Histol Jpn 43(4):347–364

Inoue M, Yagishita S, Ryo M, Hasegawa K, Amano N, Matsushita M (1997) The distribution and dynamic density of oligodendroglial cytoplasmic inclusions (GCIs) in multiple system atrophy: a correlation between the density of GCIs and the degree of involvement of striatonigral and olivopontocerebellar systems. Acta Neuropathol (Berl.) 93:585–591. https://doi.org/10.1007/s004010050655

Isacson O, Riche D, Hantraye P, Sofroniew MV, Maziere M (1989) A primate model of Huntington's disease: cross-species implantation of striatal precursor cells to the excitotoxically lesioned baboon caudate-putamen. Exp Brain Res 75(1):213–220. https://doi.org/10.1007/BF00248544

Jenner P, Marsden CD (1986) The actions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in animals as a model of Parkinson's disease. J Neural Transm Suppl 20:11–39

Jyothi HJ, Vidyadhara DJ, Mahadevan A, Philip M, Parmar SK, Manohari SG, Shankar SK, Raju TR, Alladi PA (2015) Aging causes morphological alterations in astrocytes and microglia in human substantia nigra pars compacta. Neurobiol Aging 36:3321–3333. https://doi.org/10.1016/j.neurobiolaging.2015.08.024

Kato S, Nakamura H, Hirano A, Ito H, Llena JF, Yen SH (1991) Argyrophilic ubiquitinated cytoplasmic inclusions of Leu-7-positive glial cells in olivopontocerebellar atrophy (multiple system atrophy). Acta Neuropathol (Berl.) 82:488–493. https://doi.org/10.1007/BF00293383

Kawamoto Y, Nakamura S, Akiguchi I, Kimura J (1999) Increased brain-derived neurotrophic factor-containing axons in the basal ganglia of patients with multiple system atrophy. J Neuropathol Exp Neurol 58:765–772. https://doi.org/10.1097/00005072-199907000-00010

Kawasaki T, Ishihara K, Ago Y, Baba A, Matsuda T (2007) Edaravone (3-Methyl-1-phenyl-2-pyrazolin-5-one), a radical scavenger, prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in the substantia nigra but not the striatum. J Pharmacol Exp Ther 322:274–281. https://doi.org/10.1124/jpet.106.119206

Kelly SM, Raudales R, He M, Lee JH, Kim Y, Gibb LG, Wu P, Matho K, Osten P, Graybiel AM, Huang ZJ (2018) Radial glial lineage progression and differential intermediate progenitor amplification underlie striatal compartments and circuit organization. Neuron 99:345–361.e4. https://doi.org/10.1016/j.neuron.2018.06.021

Khundakar A, Morris C, Oakley A, Thomas AJ (2011) Morphometric analysis of neuronal and glial cell pathology in the caudate nucleus in late-life depression. Am J Geriatr Psychiatry 19:132–141. https://doi.org/10.1097/JGP.0b013e3181df4642

Knott C, Stern G, Wilkin GP (2000) Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Mol Cell Neurosci 16:724–739. https://doi.org/10.1006/mcne.2000.0914

Knott C, Stern G, Kingsbury A, Welcher AA, Wilkin GP (2002) Elevated glial brain-derived neurotrophic factor in Parkinson's disease nigra. Parkinsonism Relat Disord 8:329–341. https://doi.org/10.1016/S1353-8020(02)00008-1

Komori T, Scheithauer BW, Anthony DC, Rosenblum MK, McLendon RE, Scott RM, Okazaki H, Kobayashi M (1998) Papillary glioneuronal tumor: a new variant of mixed neuronal-glial neoplasm. Am J Surg Pathol 22(10):1171–1183

Kordower JH, Goetz CG, Freeman TB, Olanow CW (1997) Dopaminergic transplants in patients with Parkinson's disease: neuroanatomical correlates of clinical recovery. Exp Neurol 144:41–46. https://doi.org/10.1006/exnr.1996.6386

Kordower JH, Goetz CG, Chu Y, Halliday GM, Nicholson DA, Musial TF, Marmion DJ, Stoessl AJ, Sossi V, Freeman TB, Olanow CW (2017) Robust graft survival and normalized dopaminergic innervation do not obligate recovery in a Parkinson disease patient. Ann Neurol 81:46–57. https://doi.org/10.1002/ana.24820

Kurkowska-Jastrzebska I, Wrońska A, Kohutnicka M, Członkowski A, Członkowska A (1999) MHC class II positive microglia and lymphocytic infiltration are present in the substantia nigra and striatum in mouse model of Parkinson's disease. Acta Neurobiol Exp (Warsz) 59:1–8

L'Episcopo F, Tirolo C, Testa N, Caniglia S, Concetta Morale M, Marchetti B (2013) Reactive astrocytes are key players in nigrostriatal dopaminergic neurorepair in the Mptp mouse model of Parkinson's disease: focus on endogenous neurorestoration. Curr Aging Sci 6:45–55

Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39:151–170. https://doi.org/10.1016/0306-4522(90)90229-W

LeVine SM, Macklin WB (1990) Iron-enriched oligodendrocytes: a reexamination of their spatial distribution. J Neurosci Res 26(4):508–512

Lewandowska E, Wierzba-Bobrowicz T, Kosno-Kruszewska E, Lechowicz W, Schmidt-Sidor B, Szpak GM, Bertrand E, Pasennik E, Gwiazda E (2004) Ultrastructural evaluation of activated forms of microglia in human brain in selected neurological diseases (SSPE, Wilson's disease and Alzheimer's disease). Folia Neuropathol 42:81–91

Lhermitte J, Kraus WM, McAlpine D (1924) Original papers: On the occurrence of abnormal deposits of iron in the brain in parkinsonism with special reference to its localisation. J Neurol Psychopathol 5(19):195

Li F, Ayaki T, Maki T, Sawamoto N, Takahashi R (2018) NLRP3 inflammasome-related proteins are upregulated in the putamen of patients with multiple system atrophy. J Neuropathol Exp Neurol 77:1055–1065. https://doi.org/10.1093/jnen/nly090

Ling ZD, Potter ED, Lipton JW, Carvey PM (1998) Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines. Exp Neurol 149:411–423. https://doi.org/10.1006/exnr.1998.6715

Lipina SJ, Colombo JA (2001) Dissociated functional recovery in parkinsonian monkeys following transplantation of astroglial cells. Brain Res 911:176–180. https://doi.org/10.1016/S0006-8993(01)02682-8

Lu SY, Pixley SK, Emerich DF, Lehman MN, Norman AB (1993) Effect of fetal striatal and astrocyte transplants into unilateral excitotoxin-lesioned striatum [WWW Document]. J Neural Transplant Plast. https://doi.org/10.1155/NP.1993.279

Ludwin SK, Kosek JC, Eng LF (1976) The topographical distribution of S-100 and GFA proteins in the adult rat brain: an immunohistochemical study using horseradish peroxidase-labelled antibodies. J Comp Neurol 165(2):197–207

Lundberg C, Horellou P, Mallet J, Björklund A (1996) Generation of DOPA-producing astrocytes by retroviral transduction of the human tyrosine hydroxylase gene: in vitro characterization and in vivo effects in the rat Parkinson model. Exp Neurol 139:39–53. https://doi.org/10.1006/exnr.1996.0079

Ma KC, Ye ZR, Fang J, Wu JV (1988) Glial fibrillary acidic protein immunohistochemical study of Alzheimer I & II astrogliosis in Wilson's disease. Acta Neurol Scand 78:290–296. https://doi.org/10.1111/j.1600-0404.1988.tb03659.x

Maswood N, Young J, Tilmont E, Zhang Z, Gash DM, Gerhardt GA, Grondin R, Roth GS, Mattison J, Lane MA, Carson RE (2004) Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. Proc Natl Acad Sci U S A 101(52):18171–18176

Matsumoto K, Nakamura T (1996) Emerging multipotent aspects of hepatocyte growth factor. J Biochem 119(4):591–600

May VEL, Ettle B, Poehler A-M, Nuber S, Ubhi K, Rockenstein E, Winner B, Wegner M, Masliah E, Winkler J (2014) α-Synuclein impairs oligodendrocyte progenitor maturation in multiple system atrophy. Neurobiol Aging 35:2357–2368. https://doi.org/10.1016/j.neurobiolaging.2014.02.028

Mendell JR, Whitaker JN (1978) Immunocytochemical localization studies of myelin basic protein. J Cell Biol 76:502–511. https://doi.org/10.1083/jcb.76.2.502

Mendez I, Dagher A, Hong M, Hebb A, Gaudet P, Law A, Weerasinghe S, King D, Desrosiers J, Darvesh S, Acorn T, Robertson H (2000) Enhancement of survival of stored dopaminergic cells and promotion of graft survival by exposure of human fetal nigral tissue to glial cell line—derived neurotrophic factor in patients with Parkinson's disease: report of two cases and technical considerations. J Neurosurg 92:863–869. https://doi.org/10.3171/jns.2000.92.5.0863

Mikol J, Vital C, Wassef M, Chappuis P, Poupon J, Lecharpentier M, Woimant F (2005) Extensive cortico-subcortical lesions in Wilson's disease: clinico-pathological study of two cases. Acta Neuropathol (Berl) 110:451–458. https://doi.org/10.1007/s00401-005-1061-1

Mitra K, Gangopadhaya PK, Das SK (2003) Parkinsonism plus syndrome–a review. Neurol India 51(2):183

Miyakawa T, Murayama E (1976) An autopsy case of the "demyelinating type" of Wilson's disease. Acta Neuropathol 35(3):235–241

Miyamoto K, Kowalska A, Hasegawa M, Tabira T, Takahashi K, Araki W, Akiguchi I, Ikemoto A (2001) Familial frontotemporal dementia and parkinsonism with a novel mutation at an intron 10+11-splice site in the tau gene. Ann Neurol 50:117–120. https://doi.org/10.1002/ana.1083

Miyazaki I, Asanuma M, Diaz-Corrales FJ, Miyoshi K, Ogawa N (2004) Direct evidence for expression of dopamine receptors in astrocytes from basal ganglia. Brain Res 1029(1):120–123

Moon M, Kim HG, Hwang L, Seo J-H, Kim S, Hwang S, Kim S, Lee D, Chung H, Oh MS, Lee K-T, Park S (2009) Neuroprotective effect of ghrelin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease by blocking microglial activation. Neurotox Res 15:332–347. https://doi.org/10.1007/s12640-009-9037-x

Morales I, Sanchez A, Rodriguez-Sabate C, Rodriguez M (2017) Striatal astrocytes engulf dopaminergic debris in Parkinson's disease: a study in an animal model. PLoS One 12:e0185989. https://doi.org/10.1371/journal.pone.0185989

Morgan TE, Xie Z, Goldsmith S, Yoshida T, Lanzrein A-S, Stone D, Rozovsky I, Perry G, Smith MA, Finch CE (1999) The mosaic of brain glial hyperactivity during normal ageing and its attenuation by food restriction. Neuroscience 89:687–699. https://doi.org/10.1016/S0306-4522(98)00334-0

Morrow CS, Porter TJ, Xu N, Arndt ZP, Ako-Asare K, Heo HJ, Thompson EAN, Moore DL (2020) Vimentin coordinates protein turnover at the aggresome during neural stem cell quiescence exit. Cell Stem Cell 26:558–568.e9. https://doi.org/10.1016/j.stem.2020.01.018

Muramatsu Y, Kurosaki R, Watanabe H, Michimata M, Matsubara M, Imai Y, Araki T (2003) Cerebral alterations in a MPTP-mouse model of Parkinson's disease–an immunocytochemical study. J Neural Transm 110(10):1129–1144

Murillo O, Moreno D, Gazquez C, Barberia M, Cenzano I, Navarro I, Uriarte I, Sebastian V, Arruebo M, Ferrer V, Bénichou B (2019) Liver expression of a MiniATP7B gene results in long – Term restoration of copper homeostasis in a Wilson disease model in mice. Hepatology 70(1):108–126

Mythri RB, Venkateshappa C, Harish G, Mahadevan A, Muthane UB, Yasha TC, Srinivas Bharath MM, Shankar SK (2011) Evaluation of markers of oxidative stress, antioxidant function and astrocytic proliferation in the striatum and frontal cortex of Parkinson's disease brains. Neurochem Res 36:1452–1463. https://doi.org/10.1007/s11064-011-0471-9

Nagatomo K, Suga S, Saitoh M, Kogawa M, Kobayashi K, Yamamoto Y, Yamada K (2017) Dopamine D1 receptor immunoreactivity on fine processes of GFAP-positive astrocytes in the substantia nigra pars reticulata of adult mouse. Front Neuroanat 11:3. https://doi.org/10.3389/fnana.2017.00003

Nagatsu T, Sawada M (2005) Inflammatory process in Parkinson's disease: role for cytokines. Curr Pharm Des 11:999–1016. https://doi.org/10.2174/1381612053381620

Nakamura S, Akiguchi I, Kimura J (1993) A subpopulation of mouse striatal cholinergic neurons show monoamine oxidase activity. Neurosci Lett 161:141–144. https://doi.org/10.1016/0304-3940(93)90279-T

Nakamura K, Mori F, Kon T, Tanji K, Miki Y, Tomiyama M, Kurotaki H, Toyoshima Y, Kakita A, Takahashi H, Yamada M, Wakabayashi K (2016) Accumulation of phosphorylated α-synuclein in subpial and periventricular astrocytes in multiple system atrophy of long duration. Neuropathology 36:157–167. https://doi.org/10.1111/neup.12243

Narasaki M (1980) Laboratory and histological similarities between Wilson's disease and rats with copper toxicity. Acta Med Okayama 34:81–90. https://doi.org/10.18926/AMO/30535

Naskar A, Mahadevan A, Philip M, Alladi PA (2019) Aging mildly affects dendritic arborisation and synaptic protein expression in human substantia nigra pars compacta. J Chem Neuroanat 97: 57–65. https://doi.org/10.1016/j.jchemneu.2019.02.001

Nelson AB, Kreitzer AC (2014) Reassessing models of basal ganglia function and dysfunction. Annu Rev Neurosci 37:117–135. https://doi.org/10.1146/annurev-neuro-071013-013916

Nirenberg MJ, Libien J, Vonsattel JP, Fahn S (2007) Multiple system atrophy in a patient with the spinocerebellar ataxia 3 gene mutation. Mov Disord 22:251–254. https://doi.org/10.1002/mds.21231

Nishimuta M, Masui K, Yamamoto T, Ikarashi Y, Tokushige K, Hashimoto E, Nagashima Y, Shibata N (2018) Copper deposition in oligodendroglial cells in an autopsied case of hepatolenticular degeneration. Neuropathology 38:321–328. https://doi.org/10.1111/neup.12456

O'Callaghan JP, Miller DB, Reinhard JF (1990) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced damage of striatal dopaminergic fibers attenuates subsequent astrocyte response to MPTP. Neurosci Lett 117:228–233. https://doi.org/10.1016/0304-3940(90)90149-4

Ogawa M, Araki M, Nagatsu I, Yoshida M (1989) Astroglial cell alteration caused by neurotoxins: immunohistochemical observations with antibodies to glial fibrillary acidic protein, laminin, and tyrosine hydroxylase. Exp Neurol 106:187–196. https://doi.org/10.1016/0014-4886(89)90093-9

Oo TF, Ries V, Cho J, Kholodilov N, Burke RE (2005) Anatomical basis of glial cell line-derived neurotrophic factor expression in the striatum and related basal ganglia during postnatal development of the rat. J Comp Neurol 484(1):57–67

Papp MI, Lantos PL (1994) The distribution of oligodendroglial inclusions in multiple system atrophy and its relevance to clinical symptomatology. Brain 117:235–243. https://doi.org/10.1093/brain/117.2.235

Papp MI, Kahn JE, Lantos PL (1989) Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome). J Neurol Sci 94:79–100. https://doi.org/10.1016/0022-510X(89)90219-0

Pasinetti GM, Finch CE (1991) Sulfated glycoprotein-2 (SGP-2) mRNA is expressed in rat striatal astrocytes following ibotenic acid lesions. Neurosci Lett 130:1–4. https://doi.org/10.1016/0304-3940(91)90213-D

Pasinetti GM, Morgan DG, Finch CE (1991) Disappearance of GAD-mRNA and tyrosine hydroxylase in substantia nigra following striatal ibotenic acid lesions: evidence for transneuronal regression. Exp Neurol 112:131–139. https://doi.org/10.1016/0014-4886(91)90063-I

Pasinetti GM, Hassler M, Stone D, Finch CE (1999) Glial gene expression during aging in rat striatum and in long-term responses to 6-OHDA lesions. Synapse 31:278–284. https://doi.org/10.1002/(SICI)1098-2396(19990315)31:4<278::AID-SYN5>3.0.CO;2-0

Pfeiffer RF (2011) Chapter 49 - Wilson's disease. In: Weiner WJ, Tolosa E (eds) Handbook of clinical neurology, Hyperkinetic movement disorders. Elsevier, Amsterdam, pp 681–709. https://doi.org/10.1016/B978-0-444-52014-2.00049-5

Poujois A, Mikol J, Woimant F (2017) Chapter 8 - Wilson disease: brain pathology. In: Członkowska A, Schilsky ML (eds) Handbook of clinical neurology, Wilson disease. Elsevier, Amsterdam, pp 77–89. https://doi.org/10.1016/B978-0-444-63625-6.00008-2

Raghavan R, de Kruijff L, Sterrenburg MD, Rogers BB, Hladik CL, White CL (2004) Alpha-synuclein expression in the developing human brain. Pediatr Dev Pathol 7:506–516. https://doi.org/10.1007/s10024-003-7080-9

Raponi E, Agenes F, Delphin C, Assard N, Baudier J, Legraverend C, Deloulme J-C (2007) S100B expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. Glia 55:165–177. https://doi.org/10.1002/glia.20445

Rataboul P, Vernier P, Biguet NF, Mallet J, Poulat P, Privat A (1989) Modulation of GFAP mRNA levels following toxic lesions in the basal ganglia of the rat. Brain Res. Bull Spinal Cord 22: 155–161. https://doi.org/10.1016/0361-9230(89)90140-8

Reed E, Lutsenko S, Bandmann O (2018) Animal models of Wilson disease. J Neurochem 146: 356–373. https://doi.org/10.1111/jnc.14323

Reiner A, Hart NM, Lei W, Deng Y (2010) Corticostriatal projection neurons – dichotomous types and dichotomous functions. Front Neuroanat 4:142. https://doi.org/10.3389/fnana.2010.00142

Reinhard JF, Miller DB, O'Callaghan JP (1988) The neurotoxicant MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) increases glial fibrillary acidic protein and decreases dopamine levels of the mouse striatum: evidence for glial response to injury. Neurosci Lett 95:246–251. https://doi.org/10.1016/0304-3940(88)90665-9

Revuelta M, Venero JL, Machado A, Cano J (1997) Deprenyl induces GFAP immunoreactivity in the intact and injured dopaminergic nigrostriatal system but fails to counteract axotomy-induced degenerative changes. Glia 21:204–216. https://doi.org/10.1002/(SICI)1098-1136(199710)21:2<204::AID-GLIA4>3.0.CO;2-6

Rouget M, Araud D, Seite R, Prochiantz A, Autillo-Touati A (1993) Astrocyte-regulated synaptogenesis: an in vitro ultrastructural study. Neurosci Lett 150:85–88. https://doi.org/10.1016/0304-3940(93)90114-Z

Rousselet A, Autillo-Touati A, Araud D, Prochiantz A (1990) In vitro regulation of neuronal morphogenesis and polarity by astrocyte-derived factors. Dev Biol 137:33–45. https://doi.org/10.1016/0012-1606(90)90005-4

Sable V, Sailaja K, Gopinath G, Tandon PN (1997) Fetal dopaminergic neurons transplanted to the normal striatum of neonatal or adult rats and to the denervated striatum of adult rats [WWW Document]. J Neural Transplant Plast. https://doi.org/10.1155/NP.1997.73

Sajin B, Steindler DA (1994) Cells on the edge: boundary astrocytes and neurons. Perspect Dev Neurobiol 2(3):275–289

Salvesen L, Ullerup BH, Sunay FB, Brudek T, Løkkegaard A, Agander TK, Winge K, Pakkenberg B (2015) Changes in total cell numbers of the basal ganglia in patients with multiple system atrophy—a stereological study. Neurobiol Dis 74:104–113. https://doi.org/10.1016/j.nbd.2014.11.008

Sanchez-Guajardo V, Annibali A, Jensen PH, Romero-Ramos M (2013) α-Synuclein vaccination prevents the accumulation of Parkinson disease-like pathologic inclusions in striatum in association with regulatory T cell recruitment in a rat model. J Neuropathol Exp Neurol 72:624–645. https://doi.org/10.1097/NEN.0b013e31829768d2

Sato T, Ando H, Tomaru U, Yoshida M, Utsunomiya A, Yamauchi J, Araya N, Yagishita N, Coler-Reilly A, Jacobson S, Yamano Y (2014) Virus-induced CXCL10-CXCR3 positive feedback loop via astrocytes is critical for maintaining chronic inflammatory lesions in HAM/TSP. Retrovirology 11(1):1–1

Sawada H, Hishida R, Hirata Y, Ono K, Suzuki H, Muramatsu S, Nakano I, Nagatsu T, Sawada M (2007) Activated microglia affect the nigro-striatal dopamine neurons differently in neonatal and aged mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J Neurosci Res 85: 1752–1761. https://doi.org/10.1002/jnr.21241

Schauwecker PE, Cogen JP, Jiang T, Cheng HW, Collier TJ, McNeill TH (1998) Differential regulation of astrocytic mRNAs in the rat striatum after lesions of the cortex or substantia nigra. Exp Neurol 149:87–96. https://doi.org/10.1006/exnr.1997.6679

Scheiber IF, Brůha R, Dušek P (2017) Pathogenesis of Wilson disease. Handb Clin Neurol 142:43–55

Schröder KF, Hopf A, Langeand H, Thörner G (1975) Morphometrisch-statistischeStrukturanalysen desStriatum, PallidumundNucleus subthalamicusbeimMenschen, Teil1 (Striatum). J Hirnforsch 16:333–350

Schwarz EJ, Alexander GM, Prockop DJ, Azizi SA (1999) Multipotential marrow stromal cells transduced to produce L-DOPA: engraftment in a rat model of Parkinson disease. Hum Gene Ther 10:2539–2549. https://doi.org/10.1089/10430349950016870

Serra PA, Sciola L, Delogu MR, Spano A, Monaco G, Miele E, Rocchitta G, Miele M, Migheli R, Desole MS (2002) The neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine induces apoptosis in mouse nigrostriatal glia relevance to nigral neuronal death and striatal neurochemical changes. J Biol Chem 277:34451–34461. https://doi.org/10.1074/jbc.M202099200

Sheng JG, Shirabe S, Nishiyama N, Schwartz JP (1993) Alterations in striatal glial fibrillary acidic protein expression in response to 6-hydroxydopamine-induced denervation. Exp Brain Res 95: 450–456. https://doi.org/10.1007/BF00227138

Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, Miller GW, Yagi T, Matsuno-Yagi A, Greenamyre JT (2003) Mechanism of toxicity in rotenone models of Parkinson's disease. J Neurosci 23:10756–10764. https://doi.org/10.1523/JNEUROSCI.23-34-10756.2003

Shimano Y, Fukuda A, Fujimoto I, Hida H, Nakajima K, Okabe A, Nishino H (1996) Tissue extract from dopamine-depleted striatum enhances differentiation of cultured striatal type-1 astrocytes. Neurosci Lett 205:193–196. https://doi.org/10.1016/0304-3940(96)12413-7

Shimoji K, Sato Y, Endoh H, Taga K, Fujiwara N, Fukuda S (1987) Relation between spinal cord and epidural blood flow. Stroke 18(6):1128–1132

Shimoji M, Pagan F, Healton EB, Mocchetti I (2009) CXCR4 and CXCL12 expression is increased in the nigro-striatal system of Parkinson's disease. Neurotox Res 16:318–328. https://doi.org/10.1007/s12640-009-9076-3

Shipp S (2017) The functional logic of corticostriatal connections. Brain Struct Funct 222:669–706. https://doi.org/10.1007/s00429-016-1250-9

Shoham S, Wertman E, Ebstein RP (1992) Iron accumulation in the rat basal ganglia after excitatory amino acid injections—dissociation from neuronal loss. Exp Neurol 118:227–241. https://doi.org/10.1016/0014-4886(92)90039-S

Smith GM, Rutishauser U, Silver J, Miller RH (1990) Maturation of astrocytes in vitro alters the extent and molecular basis of neurite outgrowth. Dev Biol 138:377–390. https://doi.org/10.1016/0012-1606(90)90204-V

Song YJC, Halliday GM, Holton JL, Lashley T, O'Sullivan SS, McCann H, Lees AJ, Ozawa T, Williams DR, Lockhart PJ, Revesz TR (2009) Degeneration in different parkinsonian syndromes relates to astrocyte type and astrocyte protein expression. J Neuropathol Exp Neurol 68:1073–1083. https://doi.org/10.1097/NEN.0b013e3181b66f1b

Stefanova N, Reindl M, Neumann M, Kahle PJ, Poewe W, Wenning GK (2007) Microglial activation mediates neurodegeneration related to oligodendroglial α – synucleinopathy: Implications for multiple system atrophy. Mov Disord 22(15):2196–2203

Stefanova N, Hainzer M, Stemberger S, Couillard-Després S, Aigner L, Poewe W, Wenning GK (2009) Striatal transplantation for multiple system atrophy—are grafts affected by α-synucleinopathy? Exp Neurol Brain Stimul Psychiatry 219:368–371. https://doi.org/10.1016/j.expneurol.2009.05.016

Steiger TK, Weiskopf N, Bunzeck N (2016) Iron level and myelin content in the ventral striatum predict memory performance in the aging brain. J Neurosci 36:3552–3558. https://doi.org/10.1523/JNEUROSCI.3617-15.2016

Streifel KM, Gonzales AL, Miranda BD, Mouneimne R, Earley S, Tjalkens R (2014) Dopaminergic neurotoxicants cause biphasic inhibition of purinergic calcium signaling in astrocytes. PLoS One 9:e110996. https://doi.org/10.1371/journal.pone.0110996

Sturrock RR (1981) Microglia in the prenatal mouse neostriatum and spinal cord. J Anat 133: 499–512

Sturrock RR (1987) Age-related changes in the number of myelinated axons and glial cells in the anterior and posterior limbs of the mouse anterior commissure. J Anat 150:111–127

Surendranathan A, O'Brien JT (2018) Clinical imaging in dementia with Lewy bodies. Evid Based Ment Health 21(2):61–65

Surgucheva I, Newell KL, Burns J, Surguchov A (2014) New α-and γ-synuclein immunopathological lesions in human brain. Acta Neuropathol Commun 2(1):1–9

Takada M, Li ZK, Hattori T (1990) Astroglial ablation prevents MPTP-induced nigrostriatal neuronal death. Brain Res 509:55–61. https://doi.org/10.1016/0006-8993(90)90308-X

Takagi S, Hayakawa N, Kimoto H, Kato H, Araki T (2007) Damage to oligodendrocytes in the striatum after MPTP neurotoxicity in mice. J Neural Transm 114:1553–1557. https://doi.org/10.1007/s00702-007-0790-9

Takeuchi A, Isobe K, Miyaishi O, Sawada M, Fan Z-H, Nakashima I, Kiuchi K (1998) Microglial NO induces delayed neuronal death following acute injury in the striatum. Eur J Neurosci 10:1613–1620. https://doi.org/10.1046/j.1460-9568.1998.00168.x

Tong J, Ang L-C, Williams B, Furukawa Y, Fitzmaurice P, Guttman M, Boileau I, Hornykiewicz O, Kish SJ (2015) Low levels of astroglial markers in Parkinson's disease: relationship to α-synuclein accumulation. Neurobiol Dis 82:243–253. https://doi.org/10.1016/j.nbd.2015.06.010

Tornatore C, Baker-Cairns B, Yadid G, Hamilton R, Meyers K, Atwood W, Cummins A, Tanner V, Major E (1996) Expression of tyrosine hydroxylase in an immortalized human fetal astrocyte cell line: in vitro characterization and engraftment into the rodent striatum. Cell Transplant 5:145–163. https://doi.org/10.1016/0963-6897(95)02041-1

Tripanichkul W, Jaroensuppaperch E (2012) Curcumin protects nigrostriatal dopaminergic neurons and reduces glial activation in 6-hydroxydopamine hemiparkinsonian mice model. Int J Neurosci 122:263–270. https://doi.org/10.3109/00207454.2011.648760

Valera E, Ubhi K, Mante M, Rockenstein E, Masliah E (2014) Antidepressants reduce neuroinflammatory responses and astroglial alpha-synuclein accumulation in a transgenic mouse model of multiple system atrophy. Glia 62:317–337. https://doi.org/10.1002/glia.22610

Vroon A, Drukarch B, Bol JGJM, Cras P, Brevé JJP, Allan SM, Relton JK, Hoogland PVJM, Van Dam A-M (2007) Neuroinflammation in Parkinson's patients and MPTP-treated mice is not restricted to the nigrostriatal system: microgliosis and differential expression of interleukin-1 receptors in the olfactory bulb. Exp Gerontol 42:762–771. https://doi.org/10.1016/j.exger.2007.04.010

Wagner J, Åkerud P, Castro DS, Holm PC, Canals JM, Snyder EY, Perlmann T, Arenas E (1999) Induction of a midbrain dopaminergic phenotype in Nurr 1-overexpressing neural stem cells by type 1 astrocytes. Nat Biotechnol 17:653–659. https://doi.org/10.1038/10862

Wakabayashi K, Oyanagi K, Makifuchi T, Ikuta F, Homma A, Homma Y, Horikawa Y, Tokiguchi S (1994) Corticobasal degeneration: etiopathological significance of the cytoskeletal alterations. Acta Neuropathol (Berl) 87:545–553. https://doi.org/10.1007/BF00293314

Wilson SAK (1912) Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain 34:295

Wu C, Wang C, Popescu DC, Zhu W, Somoza EA, Zhu J, Condie AG, Flask CA, Miller RH, Macklin W, Wang Y (2010) A novel PET marker for in vivo quantification of myelination. Bioorg Med Chem 18:8592–8599. https://doi.org/10.1016/j.bmc.2010.10.018

Youdim MBH, Riederer P (1993) The relevance of glial monoamine oxidase-B and polyamines to the action of selegiline in Parkinson's disease. Mov Disord 8:S8–S13. https://doi.org/10.1002/mds.870080504

Zhou FC, Buchwald N (1989) Connectivities of the striatal grafts in adult rat brain: a rich afference and scant striatonigral efference. Brain Res 504:15–30. https://doi.org/10.1016/0006-8993(89)91592-8

Interplay Between Microglia and Astrocytes During Neuroinflammation: Lessons Learnt from In Vitro and In Vivo Models of Sporadic Amyotrophic Lateral Sclerosis

Pooja Shree Mishra, Anu Mary Varghese, K. Vijayalakshmi, Veeramani Preethish-Kumar, Kiran Polavarapu, Seena Vengalil, Atchayaram Nalini, Phalguni Anand Alladi, Talakad N. Sathyaprabha, and Trichur R. Raju

Abstract

The etiopathogenesis of amyotrophic lateral sclerosis (ALS) remains unexplained with no effective cure, despite years of dedicated research. It can be attributed to the lack of proper diagnosis at early stages, complex multifactorial pathology of ALS and the accentuated disease progression. There is an urgent need for suitable biomarkers to diagnose the disease efficiently at an early stage, as well as early therapeutic interventions to treat and/or reverse the neurodegeneration in ALS. Consequently, understanding the underlying disease pathomechanism becomes critical.

Non-cell autonomous disease progression and neurodegeneration through glia-mediated neuroinflammation has come to be recognized as a plausible explanation for the etiopathogenesis and, more importantly, for developing new therapeutic avenues. Incidentally, most of the current understanding of ALS comes from the animal models designed with genetic mutations/overexpression

P. S. Mishra
CERVO Brain Research Center, Quebec City, QC, Canada

A. M. Varghese · K. Vijayalakshmi · T. N. Sathyaprabha · T. R. Raju (✉)
Department of Neurophysiology, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, India

V. Preethish-Kumar · K. Polavarapu · S. Vengalil · A. Nalini
Department of Neurology, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, India

P. A. Alladi
Department of Clinical Psychopharmacology & Neurotoxicology, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_16

to recapitulate the disease symptoms of the familial form, thus precluding their relevance to the sporadic ALS (SALS) pathogenesis. In this chapter, we will explore the clinical biomarkers for diagnosis, as well as the molecular mechanism of glia-mediated neuroinflammation in SALS and associated therapeutic avenues.

Keywords

Motor neuron · Astrocytes · Microglia · Neuroinflammation · ALS biomarker

1 Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating and progressive degenerative disorder of motor neurons, which leads to muscular weakness, fasciculation, dysphagia and dysarthria and ultimately results in the paralysis of respiratory muscles and death. Majority (~90%) of the cases are sporadic with no known cause (sALS), and the remaining 10% of the cases are inherited (familial ALS, fALS). The fALS cases and approximately 1–5% of sALS cases are mapped to mutations in several genes including SOD-1, TDP-43, FUS/TLS, C9ORF72, senataxin/SEXT, Optineurin/OPTN, VCP/VCP, angiogenin/ANG, VAPB/VAPB, TBK1, NEK1 and ubiquilin (Al-Chalabi et al. 2017; Gurney et al. 1994; Kenna et al. 2016; McGoldrick et al. 2013; Nguyen et al. 2018; Renton et al. 2014).

Technological advances in sequencing methods have led to the discovery of several new genes in the recent years including KIF5A/Kinesin Family Member 5A, ANXA11/Annexin A11, GLT8D1/Glycosyltransferase 8 domain-containing 1 and TIA1/cytotoxic granule-associated RNA binding protein [reviewed by Brenner and Weishaupt (2019)].

Both forms are often clinically indistinguishable and also show the same neuropathological pattern suggesting an overlap of at least the downstream pathologic pathways. The downstream pathways of most of the genes target RNA metabolism, DNA repair autophagy as well as axonal transport (Brenner and Weishaupt 2019; Weishaupt et al. 2016).

Investigations conducted on the animal models based on these genetic mutations have indicated several mechanisms implicated in the neurodegeneration observed in ALS. These include organellar dysfunction, glutamate excitotoxicity, blood-brain barrier (BBB) and/or blood-spinal cord barrier (BSCB) impairment and oxidative stress (Mancuso and Navarro 2015).

In addition to the cellular pathology of motor neurons, the non-cell autonomous pathology and inflammatory cascades observed in the autopsy studies as well as genetic models implicate a crucial contribution of glia in ALS pathology (Dibaj et al. 2011; Julien 2007; Puentes et al. 2016). However, most of this current understanding stems from genetic models, which questions their relevance to the predominant sALS variant. In this chapter, we will focus on neuroinflammation and the toxic role played by both astrocytes and microglia leading to the disease pathology from the perspective of sALS.

2 Lessons from the Model of sALS

Several model systems to understand fALS pathogenesis are available due to the ease with which the transgenic models can be developed unlike the sALS. Therefore, fewer studies have been conducted on sALS. Cerebrospinal fluid from the patients suffering from sALS (ALS-CSF) has been utilized to develop cellular/animal models for sALS. Cellular models were developed by exposing motor neuronal cell line (NSC-34 cells), mixed primary cultures from spinal cord of embryonic and neonatal rat pups, primary glial cultures (microglia and astrocytes) and motor neurons derived from stem cells to ALS-CSF (Shahani et al. 2001; Shobha et al. 2010; Sumitha et al. 2019; Vijayalakshmi et al. 2009). Animal models were developed by administering ALS-CSF intrathecally into rat neonates or intracerebroventricularly into adult rats or mice (Mishra et al. 2020; Shanmukha et al. 2018; Rao et al. 1995; Sankaranarayani et al. 2010, 2014; Shahani et al. 2004). The ALS-CSF model thus mimics the sALS pathology efficiently, in terms of morphological and functional alterations in motor neurons and glia, as demonstrated by several molecular, immunohistological, electron microscopic, electrophysiological and behavioural investigations. ALS-CSF can cause aberrant phosphorylation of neurofilaments, a decrease in the expression of voltage-gated ion channels like Kv1.6 and Nav1.6, organellar abnormalities including Golgi and endoplasmic reticulum (ER) fragmentation, mitochondrial and lysosomal dysfunction as well as diminished protein and m-RNA levels of trophic factors (Shahani et al. 2001, 2004; Sumitha et al. 2019; Vijayalakshmi et al. 2009, 2011, 2015; Rao et al. 1995; Deepa et al. 2011; Gunasekaran et al. 2009; Kulshreshtha et al. 2011; Ramamohan et al. 2007; Sharma et al. 2016).

Administration of ALS-CSF in the adult rats resulted in abnormal electrophysiological activity of the motor neurons. The motor and cognitive performance in the adult rodent models was also impacted as determined by rotarod, grip strength, open field and memory impairment tests (Mishra et al. 2020; Sankaranarayani et al. 2010, 2014).

3 Circulating Pathology: Does CSF Hold Clues?

There is an increasing consensus on the possibility of altered CSF dynamics, its potential to seed and propagate ALS as well as its potential in determining disease progression and neurotherapeutics avenues (Ng Kee Kwong et al. 2020; Smith et al. 2015). Evidently, proteomic analysis of the ALS-CSF showed an upregulation of various glial secreted proteins including chitotriosidase (Chit-1), chitinases 3 like protein-1 and 2 and osteopontin, suggesting the role of glia in sALS pathology (Varghese et al. 2013, 2020) (Fig. 1).

It is highly possible that under the influence of toxic factors in ALS-CSF, glia undergoes morphological transformation to attain toxic phenotype and release more neurotoxic factors and less trophic factors leading to intense neuroinflammation and ultimately death of motor neurons (Lewis et al. 2012).

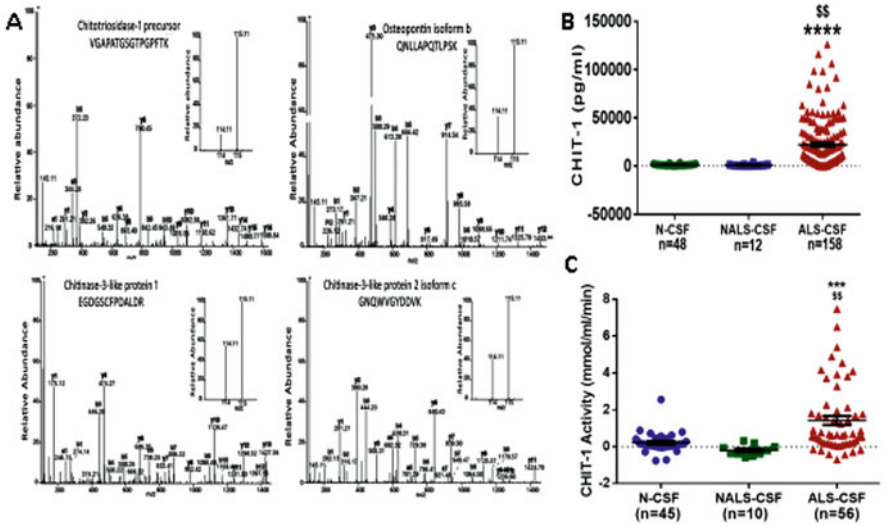

Fig. 1 Upregulated proteins in ALS-CSF. (**a**) Representative MS/MS spectra of the peptides of the upregulated proteins in ALS-CSF, namely, CHIT-1, CHI3L1 and CHI3L2 and osteopontin (Adapted from Varghese et al. 2013). (**b**, **c**) Increased levels, as well as the enzyme activity of CHIT-1 in ALS-CSF compared to controls (Adapted from Varghese et al. 2020)

4 Neuroinflammation in sALS

Neuroinflammation is a protective response targeted towards any insult to the central nervous system (CNS) and involves microglia and astrocytes as the first line of defence. The glial cells become active and undergo structural and functional changes to show graded response depending on the site, duration and severity of the insult (Fig. 2). Through inflammation, these cells survey, defend and scavenge cellular and tissue damage via mechanisms like antigen presentation, phagocytosis or recruitment of the adaptive immunity (Lewis et al. 2012; Hanisch and Kettenmann 2007). Acute inflammation is temporary and beneficial, where homeostasis is maintained after the initial glial activation and rescue. In the chronic state, however, the balance is generally lost, and it results in a prolonged inflammation that culminates in the damage to the CNS (Allaman et al. 2011; Hooten et al. 2015). The specific role of astrocytes and microglia in the neuroinflammation associated with sALS is discussed below.

4.1 Astrocytes

Astrocytes are ectodermal cells that provide nutritional and trophic support to neurons, as well as regulate excitotoxicity.

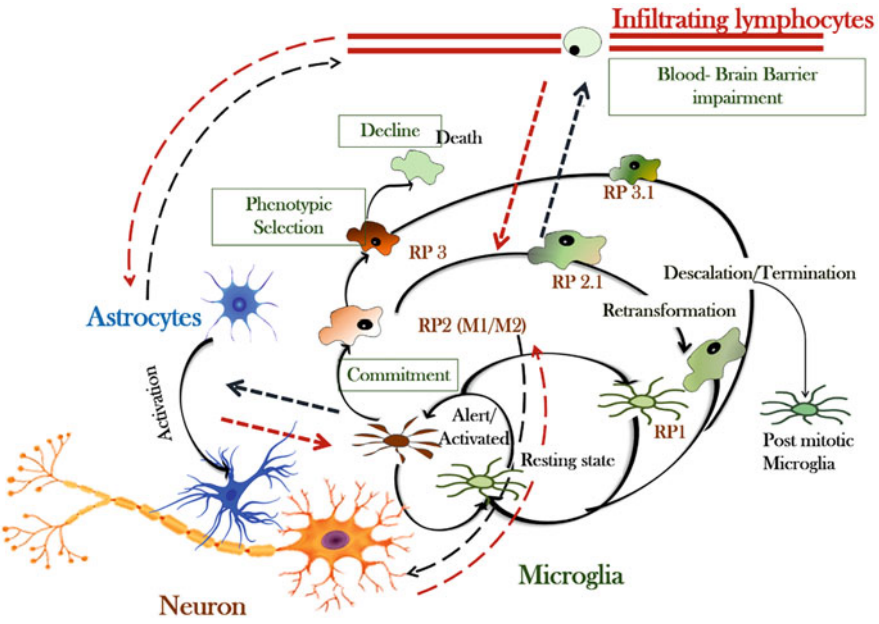

Fig. 2 Summary of neuroinflammation and the interaction between neurons, astrocytes and microglia. Note the structural and functional dynamicity of microglial activity states throughout an activation process (RP: reactive phenotype). RP1 denotes microglial priming, and RP2 denotes commitment to the activated state (alternate or classical), which can either transform into a reversible phenotype RP 2.1 or to RP 3. RP can further undergo irreversible transformation leading to death or 3.1 leading to reversible transformation. In chronic neuroinflammation, the process is imbalanced favouring events like blood-brain/spinal cord barrier impairments and active recruitment of adaptive immunity

Exposure to ALS-CSF caused activation of astrocytes. Activated astrocytes have been reported to transform from a protoplasmic morphology to a process bearing, fibrous form. This is concomitant with enhanced expression of glial fibrillary acidic protein (GFAP) and S100β (Shobha et al. 2010). These process-bearing fibrous astrocytes contribute to inflammation as evident through observations of increased pro-inflammatory cytokines in the process bearing astrocytes.

The activated astrocytes can either be neurotoxic (A1 astrocytes) or neuroprotective (A2 astrocytes) depending on the stimulus, and this process is termed as glial polarization which is guided by the microenvironment surrounding the astrocytes (Carpentier et al. 2005; Jang et al. 2013; Li et al. 2019). Neuroprotective role of astrocytes has been proposed in stroke and spinal cord injury (Sofroniew 2005). Lepore and colleagues reported an amelioration of the disease symptoms, with reduction in microgliosis following transplantation of astroglial precursor cells into the spinal cord of mSOD-1 mice, to generate healthy astrocyte pools (Lepore et al. 2008). Investigations employing human iPSC-derived astrocytes from ALS patients and chimeric mSOD1/TDP-43 models also corroborate the

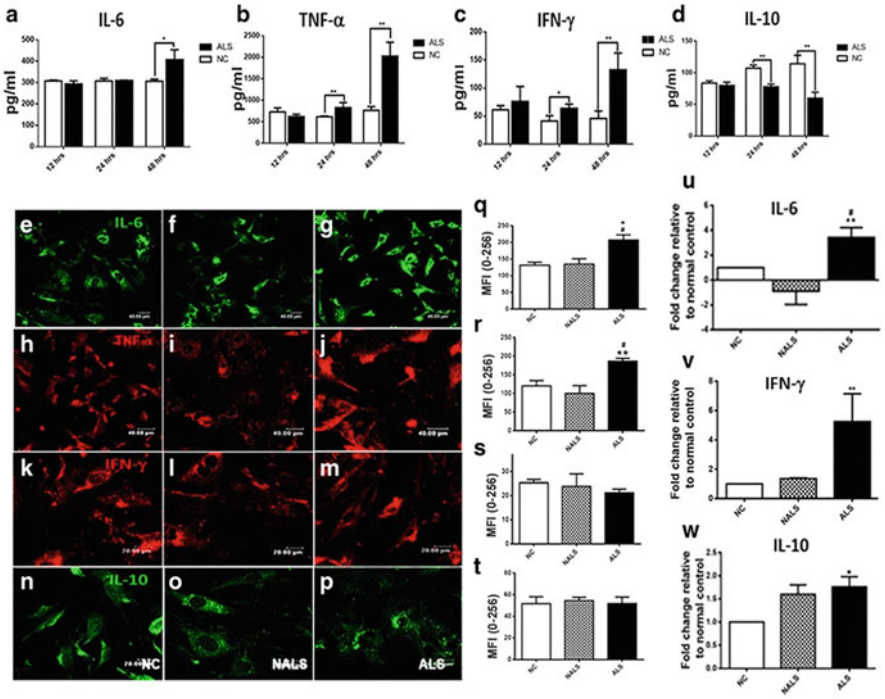

Fig. 3 ALS-CSF triggers synthesis and release of the astroglial pro-inflammatory cytokines, while anti-inflammatory cytokine IL-10 is downregulated at the protein as well as m-RNA levels. (Adapted from Mishra et al. 2016)

possible toxicity of astrocytes in disease initiation and progression (Haidet-Phillips et al. 2011; Meyer et al. 2014).

Toxic astrocytes can enhance oxidative stress through reactive oxygen species (ROS), excess glutamate and nitric oxide (NO) production, which has been known to cause DNA damage (Swarnkar et al. 2012; Mishra et al. 2016). Astrocyte toxicity has also been observed in response to inflammatory cytokines, as well as the free radicals released by M1 microglia (Zhao et al. 2013). In addition to enhanced expression of S100β and GFAP, activated toxic astrocytes can regulate trophic, inflammatory and/or toxic responses (Mishra et al. 2016). The upregulation of mRNA and protein levels of various cytokines including interferon gamma (IFN-γ), interleukin-6 (IL-6), and tumour necrosis factor (TNF)-α was also reported in response to ALS-CSF. These findings indicate induction of a vicious cycle by the inflammatory factors in ALS-CSF, including CHIT-1, and other chitinases, as well as osteopontin, via synthesis and secretion of pro-inflammatory cytokines. The temporal patterns of cytokine release in these investigations (Fig. 3) reflect overexpression of IFN-γ and TNF-α peaking at 24 h, while that of IL-6 at 48 h, suggesting that IL-6 upregulation occurs as the downstream event. Investigations have revealed that in addition to inducing inflammatory signalling, IFN-γ and TNF-α

could induce oxidative stress in the presence of inflamed glial cells (Mir et al. 2009). IL-6 can mediate sustained chronic inflammation and induce a shift from innate to adaptive immunity (Kaplanski et al. 2003). Moreover, ALS-CSF exposure resulted in reduced levels of IL-10, the anti-inflammatory cytokine (Fig. 3). Expression of IL-10 in spinal cord extended the survival of SOD1-G93A mice (Gravel et al. 2016). Decreased IL-10 expression in astrocytes suggests their inability to repair the ALS-CSF-induced neurotoxic insult.

Moreover, pro-inflammatory cytokines are known to exacerbate glutamatergic toxicity by inhibiting astroglial glutamate transporters and also by increasing the expression of glutamate receptors at the synapses (Hu et al. 2000; Pickering et al. 2005). Downregulation of the astroglial glutamate transporter, GLT-1 expression, was induced by ALS-CSF in spinal cord cultures as well as lumbar spinal cords of neonatal rat pups that results in reduced glutamate uptake (Shobha et al. 2007). In addition to the reduction in glutamate uptake, increased astroglial glutamate release in response to ALS-CSF could mimic the effects of micro-vesicular release of glutamate from astrocytes as reported in response to CNS insult (Mishra et al. 2016; Bergersen and Gundersen 2009). ALS-CSF also caused elevated levels of cyclo-oxygenase 2 (COX-2) and prostaglandin E2 (PGE-2) in activated astrocytes, which could further aggravate the degeneration of motor neurons. PGE-2, ROS and NO can also promote glutamate release (Bal-Price and Brown 2001; Socodato et al. 2015). It can act through purinergic receptors and Ca^{2+} wave propagation (Burnstock and Boeynaems 2014; Glaser et al. 2013) or induce necrosis and necroptosis that has previously been reported upon exposure of healthy motor neurons to ALS-CSF (Vijayalakshmi et al. 2009, 2015). Excess glutamate might result in neuroinflammation through inflammatory modulators including PGE2, ROS and NO (Bal-Price and Brown 2001; Vesce et al. 2007). Increased levels of astroglial NO and iNOS, as well as ROS, triggered by ALS-CSF, can also accentuate oxidative stress and reduce the glutathione (GSH) levels (Vargas et al. 2011). NO can also induce apoptosis or "necroptosis", alongside the conventional damage associated with free radicals (Günzle et al. 2016; Semmler et al. 2005; Sun et al. 2016). NO may also regulate post-translational modifications which can affect the synaptic transmission and vesicular release (Bavencoffe et al. 2014; Bradley and Steinert 2016). Excess glutamate in the synaptic cleft by constantly stimulating the postsynaptic neuron (motor neuron) can bring about excitotoxicity.

The potential of the astroglial conditioned media to exert toxicity to neurons is the resultant of propagation of ALS-like pathology through circulating fluids (ALS-CSF) (Mishra et al. 2016). In addition to the inflammatory and toxic alterations, a downregulation of the neurotrophic factors, vascular endothelial growth factor (VEGF) as well as glial cell line-derived neurotrophic factor (GDNF) was also observed, which are important trophic factors for the survival of motor neurons (Vijayalakshmi et al. 2015; Henderson et al. 1994). This downregulation of astroglial trophic factors suggests a decreased trophic support in the inflammation-mediated neurodegeneration.

4.2 Microglia

The resident immune cells of the CNS, microglia, dynamically survey and regulate the neuronal milieu in health and disease (Ferreira and Bernardino 2015). Depending on the type and nature of insult, as well as the signals from other cell types including neurons, astrocytes and infiltrating lymphocytes, microglial cells can undergo morphological and functional transformation to adopt distinct phenotypes (Fig. 2). These various phenotypes perform distinct functions ranging from triggering neuroinflammation to promoting an anti-inflammatory, healing response following an insult (Graeber and Streit 2010; Nagayach et al. 2016). Disbalance among these distinct phenotypes could lead to several neurological disorders (Cherry et al. 2014). Microglia have typically been classified in terms of polarization, namely, M1 and M2 phenotypes. However, this concept has been challenged and remains controversial (Ransohoff 2016). Microglia present in autopsy samples and genetic models, as well as in the neuroimaging studies conducted on ALS subjects, have been reported to acquire M1 phenotype (Chio et al. 2014; Chiu et al. 2013; Lloyd et al. 2000; Corcia et al. 2012). In ALS, microglial activation is generally considered neurotoxic (Borchelt 2006; Liu et al. 2009). However, studies also suggest that microglia can be neuroprotective or may not accentuate neurodegeneration in ALS models (Gowing et al. 2008; Kawamura et al. 2012). However, most of these investigations were carried out in SOD-1 model, which is not the case in the sporadic ALS (>1%) (Rosen 1993). Microglial impact in sALS has been investigated by studying their response to ALS-CSF and CHIT-1 (Varghese et al. 2020; Mishra et al. 2017).

These investigations reported dynamic microglial responses and various morphologies from the ramified to amoeboid (Fig. 4a–d″, adapted from Mishra et al. 2017). The etiogenic factors present in ALS-CSF resulted in an increased number of cells with a motile, phagocytic phenotype (Fig. 4g, i; (Mishra et al. 2017)).

Further, to understand the toxic role of microglia, the expression patterns of inflammatory molecules (IFN-γ, IL-6, TNF-α and IL-10), as well as trophic factors including GDNF and VEGF, were studied along with PGE-2, COX-2, ROS, NO and glutamate (Fig. 5). ALS-CSF caused increased expression of various pro-inflammatory molecules and oxidative stress markers, while there was a reduction in anti-inflammatory molecules and growth factors (Mishra et al. 2017).

Microglial activation is reflected by dynamically regulated morphological and functional changes. The pro-inflammatory nature of the ALS-CSF-induced microglial transformation suggests toxic microenvironment in sALS. Moreover, the toxicity of conditioned medium from microglial cultures exposed to ALS-CSF towards NSC-34 cells suggests a non-cell autonomous pathomechanism of the propagation of sALS (Mishra et al. 2017). Moreover, the study also reported release of microglial micro-vesicles (MV) which is in line with the MV release reported in reactive astrocytes, as well as in gliomas, indicating their plausible role in intercellular communication (Al-Nedawi et al. 2008; Falchi et al. 2013). Microglial MVs release in neuroinflammation could be responsible for the spread of neurotoxicity via proteins and/or microRNAs (Tang et al. 2016; Wu et al. 2015). Incidentally, the time

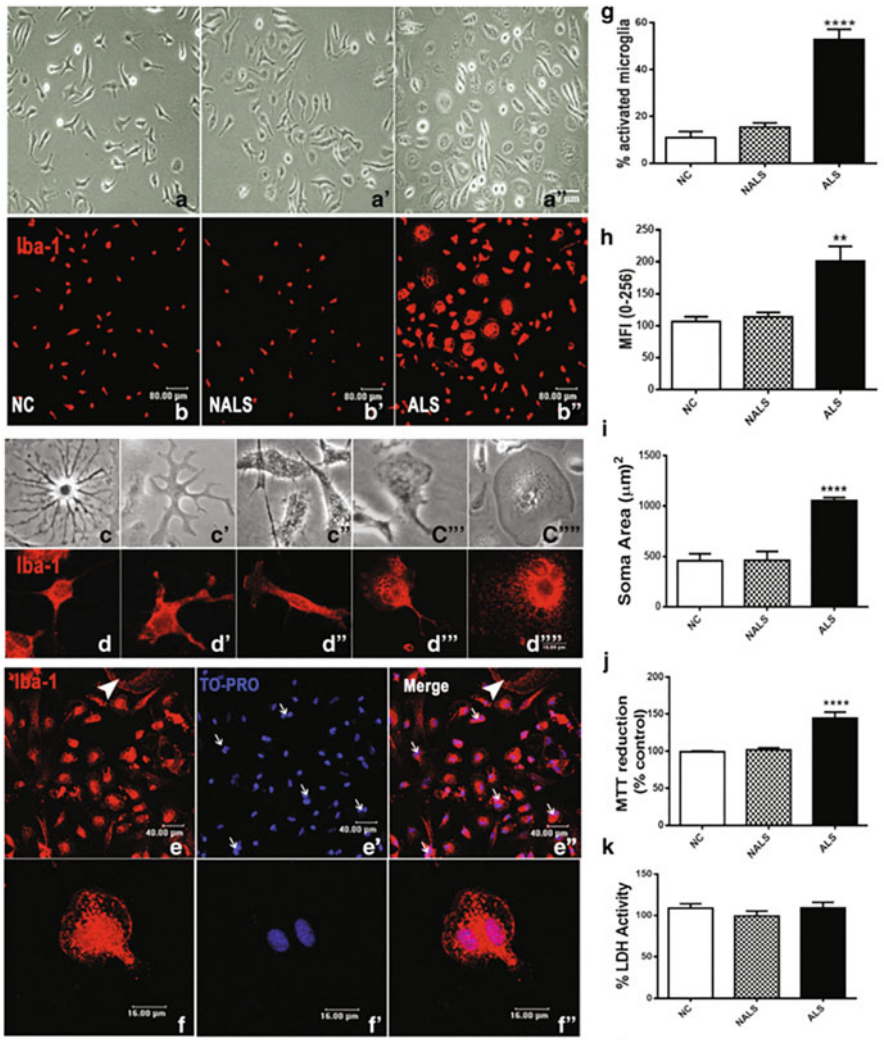

Fig. 4 ALS-CSF activates microglia. Representative phase contrast, as well as confocal images of primary microglial cultures showing morphological transformation from the ramified (resting) to various intermediate and fully activated microglial morphologies (panels **a**–**f**). (**g**) Represents increased number of migroglia; (**h**) represents increased IBA-1 expression, while (**i**) represents increased soma area in cultures exposed to ALS-CSF. Increased viability in response to ALS-CSF in the MTT assay (**j**) further corroborated the observations of microglial proliferation. Note that the LDH activity in response to ALS-CSF remains unaltered (**k**). (Adapted from Mishra et al. 2017)

of enhanced microglial MV release (12 h) coincided with that of the pro-inflammatory cytokine release from the microglial cells in response to ALS-CSF. ALS-CSF exposure also resulted in multi-nucleation and cystorrhexis in the microglial cultures at ~48 h (Mishra et al. 2017), similar to the observations in

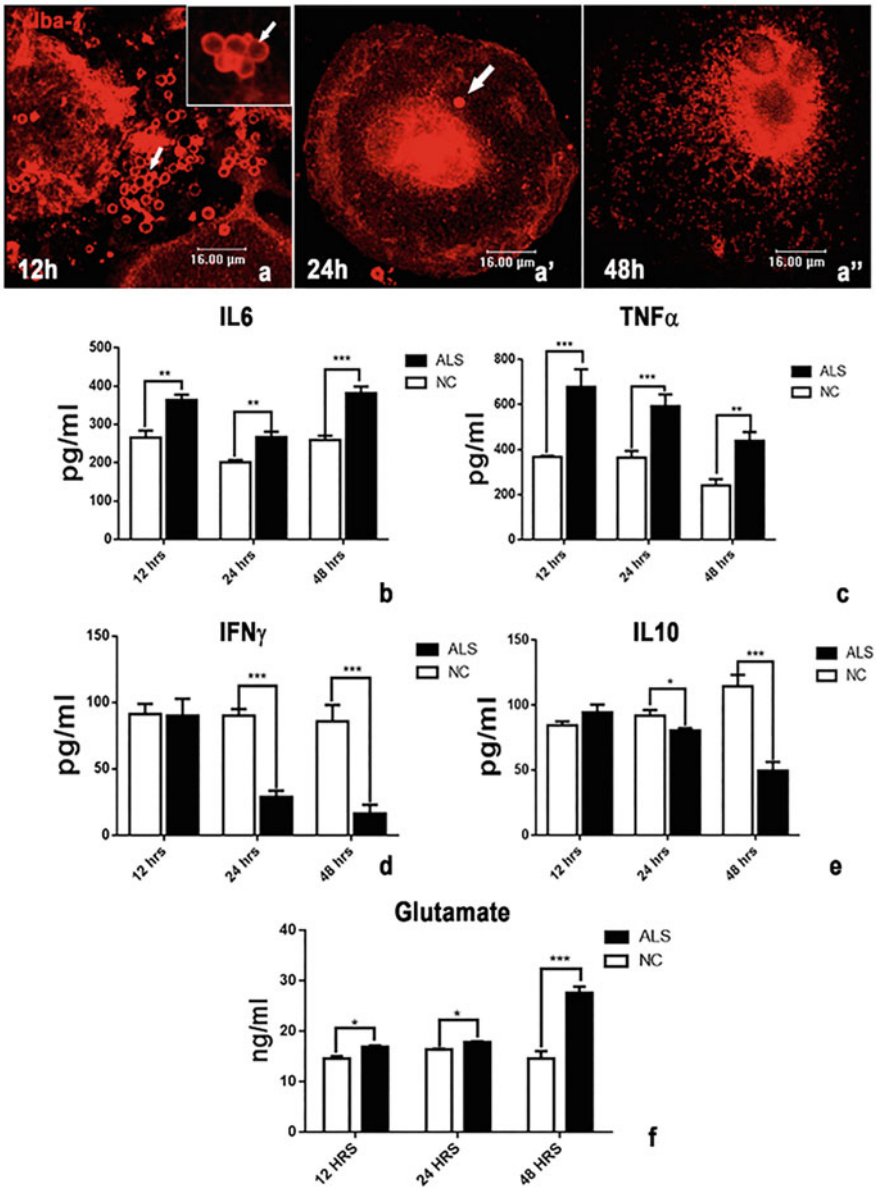

Fig. 5 Microglial activation in response to ALS-CSF is regulated temporally, structurally and functionally. ALS-CSF-induced formation of micro-vesicular structures in primary microglial cultures exposed for 12 h (panel **a**). Concomitantly, increased glutamate and cytokine expression ensued following ALS-CSF exposure (**b**–**f**) that fluctuated from 12 to 48 h, indicating dynamic microglial inflammation patterns and toxicity. (Adapted from Mishra et al. 2017)

the mutant SOD1 mice model, due to intense neuroinflammation (Fendrick et al. 2007). Studies on C9orf72 pathology also corroborate a potential role of the microglia in exacerbating neuroinflammation (Lall and Baloh 2017).

Increased expression of inflammatory mediators from microglial cells along with the withdrawal of neurotrophic support occurs following exposure to ALS-CSF (Mishra et al. 2017). Overexpression of iNOS and reduced levels of arginase may further indicate induction of activated (M1) microglia (Lewis et al. 2012). This is corroborated by the increased secretion of TNF-α and IL-6, as well as reduced release of the microglial cytokines IL-10 and IFN-γ which are known to play a neuroprotective role (Mishra et al. 2017). Investigations on the mutant SOD-1 mice model further indicate a microglial profile specific to ALS (Chiu et al. 2013; Nikodemova et al. 2014). These observations might also suggest a possible switch between the neurotoxic, pro-inflammatory and the neuroprotective, anti-inflammatory phenotypes of microglia that is reversible and dynamically regulated.

Activated microglia downregulates astroglial glutamate transporter, via release of glutamate (Takaki et al. 2012). The increased release of glutamate by microglia and astroglia can cause neurodegeneration as described earlier. The neurotoxicity exerted by the microglial conditioned media in response to ALS-CSF (ALS-MCM) clearly shows the pro-inflammatory role microglial secretions. The downregulation of microglial IFN-γ secretion suggests a reduced anti-inflammatory response (Mishra et al. 2017). Downregulation of microglial VEGF is also a hallmark of phagocytic microglial phenotype (Mishra et al. 2017; Margaritescu et al. 2011). A significant reduction in GDNF expression has previously been reported in response to LPS, suggesting that it could be a feature of inflammatory process (Matsushita et al. 2008).

Although microglial activation occurs early, major degenerative changes in the neurons were observed at 48 h (Vijayalakshmi et al. 2009; Mishra et al. 2017). In addition, prominent changes to astroglial physiology in response to ALS-CSF, including gliosis or cytokine secretion, were not observed before 48 h (Mishra et al. 2016). The inflammatory behaviour of astrocytes suggests a synergism between astroglia and microglia, in accentuating, maintaining and exacerbating ALS-CSF-mediated insult (Vijayalakshmi et al. 2009; Mishra et al. 2017). The potential of activated microglia in inducing the transformation of A2 astrocytes to A1 astrocytes through secretion of factors like TNF-α, IL-1 and C1q has been suggested. On the one hand, A1 astrocytes fail to promote microglial phagocytosis, neuronal survival and synaptogenesis and on the other induce neuronal and oligodendrocytic death (Liddelow et al. 2017).

5 Chitotriosidase as the Neuroinflammatory Marker in ALS

CHIT-1, the protein reported to be primarily upregulated in ALS-CSF, is known to be a marker of chronic activation of peripheral macrophages (Ramanathan et al. 2013). However, its function in the CNS pathology is unclear. While a pro-inflammatory potential of CHIT-1 has been reported in stroke (Di Rosa et al.

2013), its neuroprotective role in multiple sclerosis has been discussed (Sotgiu et al. 2008). The cell-specific upregulation of CHIT-1 in microglial exposed to ALS-CSF (Varghese et al. 2013) highlights the pivotal role of chronically activated microglia in eliciting neuroinflammatory response in ALS patients. Furthermore, the selectivity of the action of CHIT-1 on microglial cells suggests that the vicious cycle of neuroinflammation seen in ALS is primarily driven by microglia and propagated through cellular communication and/or circulating biological fluids (Varghese et al. 2013, 2020; Mishra et al. 2017). Since the primary source of reactive microglial pool in ALS is endogenous to the CNS and not derived from the circulating peripheral monocytes (Chiu et al. 2013), the resident microglial population appear to be the primary source of CHIT-1 found in ALS-CSF. This renders endogenous microglia a potential target for therapeutic approaches in the treatment of ALS.

6 Conclusion

Although the toxic molecules within the CSF can directly affect the neurons, neuroinflammation can accentuate its toxic effects on neurons. It is initiated by phenotypic activation and self-propagation of glial cells, which ultimately leads to enhanced secretion of inflammatory molecules. A much delayed and failed attempt at adopting M2 phenotype in order to stall the inflammation further exacerbates the pathology. Microglial propagation of inflammation appears to be aided by MVs, while phagocytic morphology justifies its role in scavenging (Fig. 6).

The astrocytes also undergo morphological transformation akin to the microglia to induce neurotoxicity. These cells lose their capacity to provide trophic support and adopt an inflammatory phenotype. The effects appear to scale up with progression in time.

Temporal analysis of the inflammatory processes provides a clear evidence that microglia impart an initial brisk response and thus might trigger the event, while the astrocytes further provide a "forward push" by producing the inflammatory and toxicity mediators in a sustained manner (Mishra et al. 2016, 2017). Although a chronological succession has been observed, both the cell types can independently induce neuroinflammation and hence play vital role in ALS pathology.

Although astrocytes and microglia synergistically act to produce augmented neuroinflammation, there is no optimal drug available to counter these inflammatory processes. For instance, in the animal models, minocycline had neuroprotective effects during the initial stages of ALS but detrimental in later stages or in higher doses (Gordon et al. 2007; Keller et al. 2011). It is imperative to delineate the specific pathways involved in astroglial and microglial-mediated inflammation to design a combined approach to target glia-mediated neuroinflammation.

CHIT-1, the major enzyme/protein component of the ALS-CSF, is microglial in origin. The neuroinflammatory action of CHIT-1 appears to be detrimental than beneficial to the neuronal health and disease progression, which further calls for detailed research in its mechanistic role and clinical implications (Varghese et al. 2020).

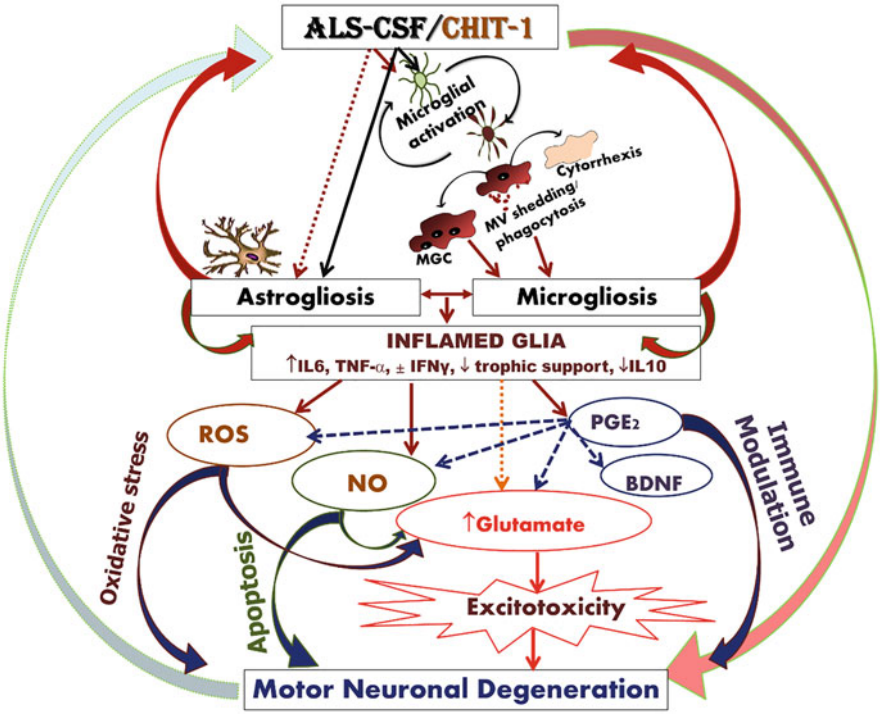

Fig. 6 ALS-CSF/CHIT-1 induced neurotoxicity involves glial responses. Microglial activation initiates early events of sALS pathophysiology, resulting in further microglial overactivation. Subsequently, astroglial cells undergo activation resulting in a sustained realize of pro-inflammatory cytokines and downregulation of beneficial trophic factors through a "push forward" mechanism. This leads to motor neuronal degeneration through accentuated neuroinflammatory responses. *MGC* multinucleated glial cells, *MV shedding/phagocytosis* activated microglia either shedding micro-vesicles or actively engaged in phagocytosis, *cystorrhexis* cytoplasmic fragmentation resulting from microglial death cascade due to over activation

References

Al-Chalabi A, van den Berg LH, Veldink J (2017) Gene discovery in amyotrophic lateral sclerosis: implications for clinical management. Nat Rev Neurol 13(2):96–104. https://doi.org/10.1038/nrneurol.2016.182

Allaman I, Belanger M, Magistretti PJ (2011) Astrocyte-neuron metabolic relationships: for better and for worse. Trends Neurosci 34(2):76–87. https://doi.org/10.1016/j.tins.2010.12.001

Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J (2008) Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol 10(5):619–624. https://doi.org/10.1038/ncb1725

Bal-Price A, Brown GC (2001) Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. J Neurosci 21(17):6480–6491

Bavencoffe A, Chen SR, Pan HL (2014) Regulation of nociceptive transduction and transmission by nitric oxide. Vitam Horm 96:1–18. https://doi.org/10.1016/B978-0-12-800254-4.00001-5

Bergersen LH, Gundersen V (2009) Morphological evidence for vesicular glutamate release from astrocytes. Neuroscience 158(1):260–265. https://doi.org/10.1016/j.neuroscience.2008.03.074

Borchelt DR (2006) Amyotrophic lateral sclerosis—are microglia killing motor neurons? N Engl J Med 355(15):1611–1613. https://doi.org/10.1056/NEJMcibr064358

Bradley SA, Steinert JR (2016) Nitric oxide-mediated posttranslational modifications: impacts at the synapse. Oxidative Med Cell Longev 2016:5681036. https://doi.org/10.1155/2016/5681036

Brenner D, Weishaupt JH (2019) Update on amyotrophic lateral sclerosis genetics. Curr Opin Neurol 32(5):735–739. https://doi.org/10.1097/wco.0000000000000737

Burnstock G, Boeynaems J-M (2014) Purinergic signalling and immune cells. Purinergic Signal 10(4):529–564. https://doi.org/10.1007/s11302-014-9427-2

Carpentier PA, Begolka WS, Olson JK, Elhofy A, Karpus WJ, Miller SD (2005) Differential activation of astrocytes by innate and adaptive immune stimuli. Glia 49(3):360–374. https://doi.org/10.1002/glia.20117

Cherry JD, Olschowka JA, O'Banion MK (2014) Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. J Neuroinflammation 11:98. https://doi.org/10.1186/1742-2094-11-98

Chio A, Pagani M, Agosta F, Calvo A, Cistaro A, Filippi M (2014) Neuroimaging in amyotrophic lateral sclerosis: insights into structural and functional changes. Lancet Neurol 13(12):1228–1240. https://doi.org/10.1016/s1474-4422(14)70167-x

Chiu IM, Morimoto ET, Goodarzi H, Liao JT, O'Keeffe S, Phatnani HP, Muratet M, Carroll MC, Levy S, Tavazoie S, Myers RM, Maniatis T (2013) A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. Cell Rep 4(2):385–401. https://doi.org/10.1016/j.celrep.2013.06.018

Corcia P, Tauber C, Vercoullie J, Arlicot N, Prunier C, Praline J, Nicolas G, Venel Y, Hommet C, Baulieu J-LJP (2012) Molecular imaging of microglial activation in amyotrophic lateral sclerosis. PLoS One 7(12):e52941

Deepa P, Shahani N, Alladi PA, Vijayalakshmi K, Sathyaprabha TN, Nalini A, Ravi V, Raju TR (2011) Down regulation of trophic factors in neonatal rat spinal cord after administration of cerebrospinal fluid from sporadic amyotrophic lateral sclerosis patients. J Neural Transm (Vienna, Austria: 1996) 118(4):531–538. https://doi.org/10.1007/s00702-010-0520-6

Di Rosa M, Malaguarnera G, De Gregorio C, D'Amico F, Mazzarino MC, Malaguarnera L (2013) Modulation of chitotriosidase during macrophage differentiation. Cell Biochem Biophys 66(2):239–247. https://doi.org/10.1007/s12013-012-9471-x

Dibaj P, Steffens H, Zschuntzsch J, Kirchhoff F, Schomburg ED, Neusch C (2011) In vivo imaging reveals rapid morphological reactions of astrocytes towards focal lesions in an ALS mouse model. Neurosci Lett 497(2):148–151. https://doi.org/10.1016/j.neulet.2011.04.049

Falchi AM, Sogos V, Saba F, Piras M, Congiu T, Piludu M (2013) Astrocytes shed large membrane vesicles that contain mitochondria, lipid droplets and ATP. Histochem Cell Biol 139(2):221–231. https://doi.org/10.1007/s00418-012-1045-x

Fendrick SE, Xue QS, Streit WJ (2007) Formation of multinucleated giant cells and microglial degeneration in rats expressing a mutant Cu/Zn superoxide dismutase gene. J Neuroinflammation 4:9

Ferreira R, Bernardino L (2015) Dual role of microglia in health and disease: pushing the balance toward repair. Front Cell Neurosci 9:51. https://doi.org/10.3389/fncel.2015.00051

Glaser T, Resende RR, Ulrich H (2013) Implications of purinergic receptor-mediated intracellular calcium transients in neural differentiation. Cell Commun Signal 11(1):12. https://doi.org/10.1186/1478-811x-11-12

Gordon PH, Moore DH, Miller RG, Florence JM, Verheijde JL, Doorish C, Hilton JF, Spitalny GM, MacArthur RB, Mitsumoto H, Neville HE, Boylan K, Mozaffar T, Belsh JM, Ravits J, Bedlack RS, Graves MC, McCluskey LF, Barohn RJ, Tandan R, Western ALSSG (2007) Efficacy of

minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. Lancet Neurol 6(12):1045–1053. https://doi.org/10.1016/S1474-4422(07)70270-3

Gowing G, Philips T, Van Wijmeersch B, Audet JN, Dewil M, Van Den Bosch L, Billiau AD, Robberecht W, Julien JP (2008) Ablation of proliferating microglia does not affect motor neuron degeneration in amyotrophic lateral sclerosis caused by mutant superoxide dismutase. J Neurosci 28(41):10234–10244. https://doi.org/10.1523/jneurosci.3494-08.2008

Graeber MB, Streit WJ (2010) Microglia: biology and pathology. Acta Neuropathol 119(1):89–105. https://doi.org/10.1007/s00401-009-0622-0

Gravel M, Béland L-C, Soucy G, Abdelhamid E, Rahimian R, Gravel C, Kriz J (2016) IL-10 controls early microglial phenotypes and disease onset in ALS caused by misfolded superoxide dismutase 1. J Neurosci 36(3):1031–1048. https://doi.org/10.1523/JNEUROSCI.0854-15.2016%J

Gunasekaran R, Narayani RS, Vijayalakshmi K, Alladi PA, Shobha K, Nalini A, Sathyaprabha TN, Raju TR (2009) Exposure to cerebrospinal fluid of sporadic amyotrophic lateral sclerosis patients alters Nav1.6 and Kv1.6 channel expression in rat spinal motor neurons. Brain Res 1255:170–179. https://doi.org/10.1016/j.brainres.2008.11.099

Günzle J, Osterberg N, Saavedra JE, Weyerbrock A (2016) Nitric oxide released from JS-K induces cell death by mitotic catastrophe as part of necrosis in glioblastoma multiforme. Cell Death Dis 7(9):e2349. https://doi.org/10.1038/cddis.2016.254

Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX et al (1994) Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science (New York, NY) 264(5166):1772–1775. https://doi.org/10.1126/science.8209258

Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, Song S, Likhite S, Murtha MJ, Foust KD, Rao M, Eagle A, Kammesheidt A, Christensen A, Mendell JR, Burghes AH, Kaspar BK (2011) Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. Nat Biotechnol 29(9):824–828. https://doi.org/10.1038/nbt.1957

Hanisch U-K, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394. https://doi.org/10.1038/nn1997

Henderson CE, Phillips HS, Pollock RA, Davies AM, Lemeulle C, Armanini M, Simmons L, Moffet B, Vandlen RA, Simpson LCSL, Koliatsos VE, Rosenthal A et al (1994) GDNF: a potent survival factor for motoneurons present in peripheral nerve and muscle. Science (New York, NY) 266(5187):1062–1064. https://doi.org/10.1126/science.7973664

Hooten KG, Beers DR, Zhao W, Appel SH (2015) Protective and toxic neuroinflammation in amyotrophic lateral sclerosis. Neurotherapeutics 12(2):364–375. https://doi.org/10.1007/s13311-014-0329-3

Hu S, Sheng WS, Ehrlich LC, Peterson PK, Chao CC (2000) Cytokine effects on glutamate uptake by human astrocytes. Neuroimmunomodulation 7(3):153–159. https://doi.org/10.1159/000026433

Jang E, Kim JH, Lee S, Kim JH, Seo JW, Jin M, Lee MG, Jang IS, Lee WH, Suk K (2013) Phenotypic polarization of activated astrocytes: the critical role of lipocalin-2 in the classical inflammatory activation of astrocytes. J Immunol 191(10):5204–5219. https://doi.org/10.4049/jimmunol.1301637

Julien JP (2007) ALS: astrocytes move in as deadly neighbors. Nat Neurosci 10(5):535–537. https://doi.org/10.1038/nn0507-535

Kaplanski G, Marin V, Montero-Julian F, Mantovani A, Farnarier C (2003) IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. Trends Immunol 24(1):25–29. https://doi.org/10.1016/s1471-4906(02)00013-3

Kawamura MF, Yamasaki R, Kawamura N, Tateishi T, Nagara Y, Matsushita T, Ohyagi Y, Kira J (2012) Impaired recruitment of neuroprotective microglia and T cells during acute neuronal injury coincides with increased neuronal vulnerability in an amyotrophic lateral sclerosis model. Exp Neurol 234(2):437–445. https://doi.org/10.1016/j.expneurol.2012.01.015

Keller AF, Gravel M, Kriz J (2011) Treatment with minocycline after disease onset alters astrocyte reactivity and increases microgliosis in SOD1 mutant mice. Exp Neurol 228(1):69–79. https://doi.org/10.1016/j.expneurol.2010.12.010

Kenna KP, van Doormaal PT, Dekker AM, Ticozzi N, Kenna BJ, Diekstra FP, van Rheenen W, van Eijk KR, Jones AR, Keagle P, Shatunov A, Sproviero W, Smith BN, van Es MA, Topp SD, Kenna A, Miller JW, Fallini C, Tiloca C, McLaughlin RL, Vance C, Troakes C, Colombrita C, Mora G, Calvo A, Verde F, Al-Sarraj S, King A, Calini D, de Belleroche J, Baas F, van der Kooi AJ, de Visser M, Ten Asbroek AL, Sapp PC, McKenna-Yasek D, Polak M, Asress S, Muñoz-Blanco JL, Strom TM, Meitinger T, Morrison KE, Lauria G, Williams KL, Leigh PN, Nicholson GA, Blair IP, Leblond CS, Dion PA, Rouleau GA, Pall H, Shaw PJ, Turner MR, Talbot K, Taroni F, Boylan KB, Van Blitterswijk M, Rademakers R, Esteban-Pérez J, García-Redondo A, Van Damme P, Robberecht W, Chio A, Gellera C, Drepper C, Sendtner M, Ratti A, Glass JD, Mora JS, Basak NA, Hardiman O, Ludolph AC, Andersen PM, Weishaupt JH, Brown RH Jr, Al-Chalabi A, Silani V, Shaw CE, van den Berg LH, Veldink JH, Landers JE (2016) NEK1 variants confer susceptibility to amyotrophic lateral sclerosis. Nat Genet 48(9):1037–1042. https://doi.org/10.1038/ng.3626

Kulshreshtha D, Vijayalakshmi K, Alladi PA, Sathyaprabha TN, Nalini A, Raju TR (2011) Vascular endothelial growth factor attenuates neurodegenerative changes in the NSC-34 motor neuron cell line induced by cerebrospinal fluid of sporadic amyotrophic lateral sclerosis patients. Neurodegener Dis 8(5):322–330. https://doi.org/10.1159/000323718

Lall D, Baloh RH (2017) Microglia and C9orf72 in neuroinflammation and ALS and frontotemporal dementia. J Clin Invest 127(9):3250–3258. https://doi.org/10.1172/jci90607

Lepore AC, Rauck B, Dejea C, Pardo AC, Rao MS, Rothstein JD, Maragakis NJ (2008) Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. Nat Neurosci 11(11):1294–1301. https://doi.org/10.1038/nn.2210

Lewis CA, Manning J, Rossi F, Krieger C (2012) The neuroinflammatory response in ALS: the roles of microglia and T cells. Neurol Res Int 2012:803701. https://doi.org/10.1155/2012/803701

Li T, Chen X, Zhang C, Zhang Y, Yao W (2019) An update on reactive astrocytes in chronic pain. J Neuroinflammation 16(1):140. https://doi.org/10.1186/s12974-019-1524-2

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Munch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541(7638):481–487. https://doi.org/10.1038/nature21029

Liu Y, Hao W, Dawson A, Liu S, Fassbender K (2009) Expression of amyotrophic lateral sclerosis-linked SOD1 mutant increases the neurotoxic potential of microglia via TLR2. J Biol Chem 284(6):3691–3699. https://doi.org/10.1074/jbc.M804446200

Lloyd CM, Richardson MP, Brooks DJ, Al-Chalabi A, Leigh PN (2000) Extramotor involvement in ALS: PET studies with the GABA(A) ligand [(11)C]flumazenil. Brain 123(Pt 11):2289–2296

Mancuso R, Navarro X (2015) Amyotrophic lateral sclerosis: current perspectives from basic research to the clinic. Prog Neurobiol. https://doi.org/10.1016/j.pneurobio.2015.07.004

Margaritescu O, Pirici D, Margaritescu C (2011) VEGF expression in human brain tissue after acute ischemic stroke. Roman J Morphol Embryol 52(4):1283–1292

Matsushita Y, Nakajima K, Tohyama Y, Kurihara T, Kohsaka S (2008) Activation of microglia by endotoxin suppresses the secretion of glial cell line-derived neurotrophic factor (GDNF) through the action of protein kinase C alpha (PKCalpha) and mitogen-activated protein kinases (MAPKS). J Neurosci Res 86(9):1959–1971. https://doi.org/10.1002/jnr.21657

McGoldrick P, Joyce PI, Fisher EM, Greensmith L (2013) Rodent models of amyotrophic lateral sclerosis. Biochim Biophys Acta 1832(9):1421–1436. https://doi.org/10.1016/j.bbadis.2013.03.012

Meyer K, Ferraiuolo L, Miranda CJ, Likhite S, McElroy S, Renusch S, Ditsworth D, Lagier-Tourenne C, Smith RA, Ravits J, Burghes AH, Shaw PJ, Cleveland DW, Kolb SJ, Kaspar BK

(2014) Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS. Proc Natl Acad Sci U S A 111(2): 829–832. https://doi.org/10.1073/pnas.1314085111

Mir M, Asensio VJ, Tolosa L, Gou-Fabregas M, Soler RM, Llado J, Olmos G (2009) Tumor necrosis factor alpha and interferon gamma cooperatively induce oxidative stress and motoneuron death in rat spinal cord embryonic explants. Neuroscience 162(4):959–971. https://doi.org/10.1016/j.neuroscience.2009.05.049

Mishra PS, Dhull DK, Nalini A, Vijayalakshmi K, Sathyaprabha TN, Alladi PA, Raju TR (2016) Astroglia acquires a toxic neuroinflammatory role in response to the cerebrospinal fluid from amyotrophic lateral sclerosis patients. J Neuroinflammation 13(1):212. https://doi.org/10.1186/s12974-016-0698-0

Mishra PS, Vijayalakshmi K, Nalini A, Sathyaprabha TN, Kramer BW, Alladi PA, Raju TR (2017) Etiogenic factors present in the cerebrospinal fluid from amyotrophic lateral sclerosis patients induce predominantly pro-inflammatory responses in microglia. J Neuroinflammation 14(1): 251. https://doi.org/10.1186/s12974-017-1028-x

Mishra PS, Boutej H, Soucy G, Bareil C, Kumar S, Picher-Martel V, Dupre N, Kriz J, Julien JP (2020) Transmission of ALS pathogenesis by the cerebrospinal fluid. Acta Neuropathol Commun 8(1):65. https://doi.org/10.1186/s40478-020-00943-4

Nagayach A, Patro N, Patro I (2016) Microglia in the physiology and pathology of brain. Proc Natl Acad Sci India B Biol Sci 86(4):781–794. https://doi.org/10.1007/s40011-015-0585-y

Ng Kee Kwong KC, Mehta AR, Nedergaard M, Chandran S (2020) Defining novel functions for cerebrospinal fluid in ALS pathophysiology. Acta Neuropathol Commun 8(1):140. https://doi.org/10.1186/s40478-020-01018-0

Nguyen HP, Van Broeckhoven C, van der Zee J (2018) ALS genes in the genomic era and their implications for FTD. Trends Genet 34(6):404–423. https://doi.org/10.1016/j.tig.2018.03.001

Nikodemova M, Small AL, Smith SM, Mitchell GS, Watters JJ (2014) Spinal but not cortical microglia acquire an atypical phenotype with high VEGF, galectin-3 and osteopontin, and blunted inflammatory responses in ALS rats. Neurobiol Dis 69:43–53. https://doi.org/10.1016/j.nbd.2013.11.009

Pickering M, Cumiskey D, O'Connor JJ (2005) Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. Exp Physiol 90(5):663–670. https://doi.org/10.1113/expphysiol.2005.030734

Puentes F, Malaspina A, van Noort JM, Amor S (2016) Non-neuronal cells in ALS: role of glial, immune cells and blood-CNS barriers. Brain Pathol (Zurich, Switzerland) 26(2):248–257. https://doi.org/10.1111/bpa.12352

Ramamohan PY, Gourie-Devi M, Nalini A, Shobha K, Ramamohan Y, Joshi P, Raju TR (2007) Cerebrospinal fluid from amyotrophic lateral sclerosis patients causes fragmentation of the Golgi apparatus in the neonatal rat spinal cord. Amyotroph Lateral Scler 8(2):79–82. https://doi.org/10.1080/08037060601145489

Ramanathan R, Kohli A, Ingaramo MC, Jain A, Leng SX, Punjabi NM, Walston JD, Fedarko NS (2013) Serum chitotriosidase, a putative marker of chronically activated macrophages, increases with normal aging. J Gerontol A Biol Sci Med Sci 68(10):1303–1309. https://doi.org/10.1093/gerona/glt022

Ransohoff RM (2016) A polarizing question: do M1 and M2 microglia exist? Nat Neurosci 19(8): 987–991. https://doi.org/10.1038/nn.4338

Rao MS, Devi MG, Nalini A, Shahani N, Raju TR (1995) Neurofilament phosphorylation is increased in ventral horn neurons of neonatal rat spinal cord exposed to cerebrospinal fluid from patients with amyotrophic lateral sclerosis. Neurodegeneration 4(4):397–401. https://doi.org/10.1006/neur.1995.0048

Renton AE, Chiò A, Traynor BJ (2014) State of play in amyotrophic lateral sclerosis genetics. Nat Neurosci 17(1):17–23

Rosen DR (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 364(6435):362. https://doi.org/10.1038/364362c0

Sankaranarayani R, Nalini A, Rao Laxmi T, Raju TR (2010) Altered neuronal activities in the motor cortex with impaired motor performance in adult rats observed after infusion of cerebrospinal fluid from amyotrophic lateral sclerosis patients. Behav Brain Res 206(1):109–119. https://doi.org/10.1016/j.bbr.2009.09.009

Sankaranarayani R, Raghavan M, Nalini A, Laxmi TR, Raju TR (2014) Reach task-associated excitatory overdrive of motor cortical neurons following infusion with ALS-CSF. J Neural Transm (Vienna) 121(1):49–58. https://doi.org/10.1007/s00702-013-1071-4

Semmler A, Okulla T, Sastre M, Dumitrescu-Ozimek L, Heneka MT (2005) Systemic inflammation induces apoptosis with variable vulnerability of different brain regions. J Chem Neuroanat 30(2):144–157. https://doi.org/10.1016/j.jchemneu.2005.07.003

Shahani N, Gourie-Devi M, Nalini A, Raju TR (2001) Cyclophosphamide attenuates the degenerative changes induced by CSF from patients with amyotrophic lateral sclerosis in the neonatal rat spinal cord. J Neurol Sci 185(2):109–118. https://doi.org/10.1016/s0022-510x(01)00479-8

Shahani N, Gourie-Devi M, Nalini A, Rammohan P, Shobha K, Harsha HN, Raju TR (2004) (−)-Deprenyl alleviates the degenerative changes induced in the neonatal rat spinal cord by CSF from amyotrophic lateral sclerosis patients. Amyotroph Lateral Scler Other Motor Neuron Disord 5(3):172–179. https://doi.org/10.1080/14660820410017037

Shanmukha S, Narayanappa G, Nalini A, Alladi PA, Raju TR (2018) Sporadic amyotrophic lateral sclerosis (SALS) - skeletal muscle response to cerebrospinal fluid from SALS patients in a rat model. Dis Model Mech 11(4):dmm031997. https://doi.org/10.1242/dmm.031997

Sharma A, Varghese AM, Vijaylakshmi K, Sumitha R, Prasanna VK, Shruthi S, Chandrasekhar Sagar BK, Datta KK, Gowda H, Nalini A, Alladi PA, Christopher R, Sathyaprabha TN, Raju TR, Srinivas Bharath MM (2016) Cerebrospinal fluid from sporadic amyotrophic lateral sclerosis patients induces mitochondrial and lysosomal dysfunction. Neurochem Res 41(5):965–984. https://doi.org/10.1007/s11064-015-1779-7

Shobha K, Vijayalakshmi K, Alladi PA, Nalini A, Sathyaprabha TN, Raju TR (2007) Altered in-vitro and in-vivo expression of glial glutamate transporter-1 following exposure to cerebrospinal fluid of amyotrophic lateral sclerosis patients. J Neurol Sci 254(1–2):9–16. https://doi.org/10.1016/j.jns.2006.12.004

Shobha K, Alladi PA, Nalini A, Sathyaprabha TN, Raju TR (2010) Exposure to CSF from sporadic amyotrophic lateral sclerosis patients induces morphological transformation of astroglia and enhances GFAP and S100beta expression. Neurosci Lett 473(1):56–61. https://doi.org/10.1016/j.neulet.2010.02.022

Smith R, Myers K, Ravits J, Bowser R (2015) Amyotrophic lateral sclerosis: is the spinal fluid pathway involved in seeding and spread? Med Hypotheses 85(5):576–583. https://doi.org/10.1016/j.mehy.2015.07.014

Socodato R, Portugal CC, Canedo T, Domith I, Oliveira NA, Paes-de-Carvalho R, Relvas JB, Cossenza M (2015) c-Src deactivation by the polyphenol 3-O-caffeoylquinic acid abrogates reactive oxygen species-mediated glutamate release from microglia and neuronal excitotoxicity. Free Radic Biol Med 79:45–55. https://doi.org/10.1016/j.freeradbiomed.2014.11.019

Sofroniew MV (2005) Reactive astrocytes in neural repair and protection. Neuroscientist 11(5):400–407. https://doi.org/10.1177/1073858405278321

Sotgiu S, Musumeci S, Marconi S, Gini B, Bonetti B (2008) Different content of chitin-like polysaccharides in multiple sclerosis and Alzheimer's disease brains. J Neuroimmunol 197(1):70–73. https://doi.org/10.1016/j.jneuroim.2008.03.021

Sumitha R, Manjunatha VM, Sabitha RK, Alladi PA, Nalini A, Rao LT, Chandrasekhar Sagar BK, Steinbusch HWM, Kramer BW, Sathyaprabha TN, Raju TR (2019) Cerebrospinal fluid from patients with sporadic amyotrophic lateral sclerosis induces degeneration of motor neurons derived from human embryonic stem cells. Mol Neurobiol 56(2):1014–1034. https://doi.org/10.1007/s12035-018-1149-y

Sun W, Bao J, Lin W, Gao H, Zhao W, Zhang Q, Leung C-H, Ma D-L, Lu J, Chen X (2016) 2-Methoxy-6-acetyl-7-methyljuglone (MAM), a natural naphthoquinone, induces

NO-dependent apoptosis and necroptosis by H_2O_2-dependent JNK activation in cancer cells. Free Radic Biol Med 92:61–77. https://doi.org/10.1016/j.freeradbiomed.2016.01.014

Swarnkar S, Singh S, Goswami P, Mathur R, Patro IK, Nath C (2012) Astrocyte activation: a key step in rotenone induced cytotoxicity and DNA damage. Neurochem Res 37(10):2178–2189. https://doi.org/10.1007/s11064-012-0841-y

Takaki J, Fujimori K, Miura M, Suzuki T, Sekino Y, Sato K (2012) L-glutamate released from activated microglia downregulates astrocytic L-glutamate transporter expression in neuroinflammation: the 'collusion' hypothesis for increased extracellular L-glutamate concentration in neuroinflammation. J Neuroinflammation 9:275. https://doi.org/10.1186/1742-2094-9-275

Tang LL, Wu YB, Fang CQ, Qu P, Gao ZL (2016) NDRG2 promoted secreted miR-375 in microvesicles shed from M1 microglia, which induced neuron damage. Biochem Biophys Res Commun 469(3):392–398. https://doi.org/10.1016/j.bbrc.2015.11.098

Vargas MR, Johnson DA, Johnson JA (2011) Decreased glutathione accelerates neurological deficit and mitochondrial pathology in familial ALS-linked hSOD1(G93A) mice model. Neurobiol Dis 43(3):543–551. https://doi.org/10.1016/j.nbd.2011.04.025

Varghese AM, Sharma A, Mishra P, Vijayalakshmi K, Harsha HC, Sathyaprabha TN, Bharath SM, Nalini A, Alladi PA, Raju TR (2013) Chitotriosidase - a putative biomarker for sporadic amyotrophic lateral sclerosis. Clin Proteomics 10(1):19. https://doi.org/10.1186/1559-0275-10-19

Varghese AM, Ghosh M, Bhagat SK, Vijayalakshmi K, Preethish-Kumar V, Vengalil S, Chevula PC, Nashi S, Polavarapu K, Sharma M, Dhaliwal RS, Philip M, Nalini A, Alladi PA, Sathyaprabha TN, Raju TR (2020) Chitotriosidase, a biomarker of amyotrophic lateral sclerosis, accentuates neurodegeneration in spinal motor neurons through neuroinflammation. J Neuroinflammation 17(1):232. https://doi.org/10.1186/s12974-020-01909-y

Vesce S, Rossi D, Brambilla L, Volterra A (2007) Glutamate release from astrocytes in physiological conditions and in neurodegenerative disorders characterized by neuroinflammation. Int Rev Neurobiol 82:57–71. https://doi.org/10.1016/S0074-7742(07)82003-4

Vijayalakshmi K, Alladi PA, Sathyaprabha TN, Subramaniam JR, Nalini A, Raju TR (2009) Cerebrospinal fluid from sporadic amyotrophic lateral sclerosis patients induces degeneration of a cultured motor neuron cell line. Brain Res 1263:122–133. https://doi.org/10.1016/j.brainres.2009.01.041

Vijayalakshmi K, Alladi PA, Ghosh S, Prasanna VK, Sagar BC, Nalini A, Sathyaprabha TN, Raju TR (2011) Evidence of endoplasmic reticular stress in the spinal motor neurons exposed to CSF from sporadic amyotrophic lateral sclerosis patients. Neurobiol Dis 41(3):695–705. https://doi.org/10.1016/j.nbd.2010.12.005

Vijayalakshmi K, Ostwal P, Sumitha R, Shruthi S, Varghese AM, Mishra P, Manohari SG, Sagar BC, Sathyaprabha TN, Nalini A, Raju TR, Alladi PA (2015) Role of VEGF and VEGFR2 receptor in reversal of ALS-CSF induced degeneration of NSC-34 motor neuron cell line. Mol Neurobiol 51(3):995–1007. https://doi.org/10.1007/s12035-014-8757-y

Weishaupt JH, Hyman T, Dikic I (2016) Common molecular pathways in amyotrophic lateral sclerosis and frontotemporal dementia. Trends Mol Med 22(9):769–783. https://doi.org/10.1016/j.molmed.2016.07.005

Wu B, Huang Y, Braun AL, Tong Z, Zhao R, Li Y, Liu F, Zheng JC (2015) Glutaminase-containing microvesicles from HIV-1-infected macrophages and immune-activated microglia induce neurotoxicity. Mol Neurodegener 10:61. https://doi.org/10.1186/s13024-015-0058-z

Zhao W, Beers DR, Appel SH (2013) Immune-mediated mechanisms in the pathoprogression of amyotrophic lateral sclerosis. J Neuroimmune Pharmacol 8(4):888–899. https://doi.org/10.1007/s11481-013-9489-x

Microglia Orchestrate Inflammation via HSP60-Driven Signalling Pathway: A Road Map of Molecular Mechanism

Stuti Mohapatra, Sriparna Mukherjee, Shalini Swaroop, and Anirban Basu

Abstract

Heat shock proteins are known as biological sensors of stress. They act as chaperons and regulate intracellular protein homeostasis by assisting protein folding and assembly. To date, apart from their classical chaperon function, heat shock proteins are enlightened as fine-tuners of neuronal inflammation and regulators of signalling pathways in neurological diseases such as Alzheimer's, Parkinson's and Huntington's diseases. The neuroprotective function of HSPs is also documented as inhibitors of apoptosis, cytoskeletal shield and immunomodulators. Heat shock protein 60 (HSP60) is a mitochondrial chaperon exerting its activities along with a co-chaperon HSP10. However, it is also found in other cellular compartments, and non-mitochondrial localizations of HSP60 carry out functions other than its chaperon activity. Microglia, on the other hand, in response to danger signals, aggravates immune response. Microglial activation has been found in a large number of pathological conditions like trauma, infection, ischemia, etc. HSP60 is employed as an indicator of CNS injury by microglial activation through TLR4-dependent pathways. Microglial stimulation by any threat leads to enhanced endogenous HSP60 expression which eventually aggravates the signalling pathways directed to escalate inflammation. In this chapter we attempt to shed light on the role played by HSP60 in microglial inflammation. We also aim to elaborate the endogenous molecular pathways

Sriparna Mukherjee and Shalini Swaroop contributed equally.

S. Mohapatra · S. Swaroop · A. Basu (✉)
National Brain Research Centre, Manesar, Haryana, India
e-mail: anirban@nbrc.ac.in

S. Mukherjee
Department of Pharmacology and Physiology, Pavilion Roger-Gaudry, Université de Montréal, Montréal, QC, Canada

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_17

inside microglia driven by HSP60 which in turn are essential for triggering off inflammatory pathways. Altogether this chapter is a brief insight into molecular mechanisms of HSP60 controlling neuroinflammation.

Keywords

Microglia · Inflammation · Heat shock protein 60 · Japanese encephalitis virus · IL-1β · NLRP3 inflammasome · TLR4

1 Introduction

Heat Shock Proteins at a Glance

Heat shock proteins (HSPs) belong to a polypeptide family, whose major function is cellular protection. These proteins are often produced by cells in high amounts in response to various stresses such as hyperthermia, oxygen radicals, heavy metals, ethanol, inflammatory diseases and viral infections and hence are known as stress proteins (Locke et al. 1990; Minowada and Welch 1995; Su and Gordon 1997; Udelsman et al. 1993). HSPs were discovered accidentally by F. M. Ritossa in 1962, in the larval salivary glands of *Drosophila melanogaster* upon inducing heat shock in flies (Ritossa 1964). Eukaryotes have heat shock genes that have heat shock elements (HSE) in their promoters. Upon exposure to thermal stress, these HSE bind to heat shock transcription factors (HSFs), and heat shock genes are actively transcribed producing HSPs (Sorger and Pelham 1987). HSPs are ubiquitously expressed in all organisms, ranging from tiny bacteria to mighty humans. They belong to a large group of highly evolutionary conserved proteins and are extensively involved in protein homeostasis by acting as chaperones. HSPs facilitate the process of protein folding and refolding, bind to unfolded proteins, prevent their aggregation and even target them for degradation if needed (Parag et al. 1987). Apart from chaperone functions, the proteins have numerous other extracellular and intracellular functions based on their cellular location. HSPs are found in cytosol as well as organelles like mitochondria, nucleus, endoplasmic reticulum and chloroplasts, implying their distinct roles inside a cell (Boston et al. 1996). It has been reported that HSPs act as immunoregulatory agents, aid in the production of cytokines (Asea et al. 2000) and also include various pro-apoptotic and anti-apoptotic proteins which interact with various other cellular proteins (Garrido et al. 1999; Buzzard et al. 1998).

2 Classification of Heat Shock Proteins (HSPs)

Heat shock proteins are further classified into many families on the basis of their molecular weights—HSP100 (100 kDa), HSP90 (90 kDa), HSP70 (70 kDa), HSP60 (60 kDa) and other small molecular weight HSPs. These HSPs are further localized

in different organelles of the cell on the basis of their function. For an ease of reference, we have listed the different heat shock proteins according to their gene families, cellular location, function and expression in Table 1.

From here on, we will focus on HSPD family (HSP60) in details for a better understanding of its role in the cellular context.

HSPD Family (HSP60)

Main member of this group is HSP60 (HSPD1), an important mitochondrial chaperone involved in many cellular activities.

HSPD1/HSP60 class of chaperone exists for mitochondrial protein folding but is also induced during stressful conditions. This family is important for mitochondrial biogenesis (Cheng et al. 1989; Voos and Röttgers 2002) as well as for maintaining the proper health and functioning of the mitochondria (Lin et al. 2001).

Structure of a typical HSPD-family member comprises of three domains—apical, intermediate and equatorial, respectively—and mostly structure-related information on HSP60 is derived from its prokaryotic homolog GroEL (Sigler et al. 1998). HSPD chaperones work in close association with members of HSPE family. The chaperone activity of HSPD family is ATP-dependent (Xu et al. 1997).

HSPD1/HSP60 is expressed endogenously in almost all cells of the brain—astrocytes, neurons, microglia, oligodendrocytes and ependymal cells (Bajramović et al. 2000; Rosenberger et al. 2015; D'Souza and Brown 1998). Most of HSPD1/HSP60 protein resides in mitochondria, but 20–40% of it is found in other extracellular sites such as cytosol or circulated in plasma (Cechetto et al. 2000; Gupta and Knowlton 2002; Soltys and Gupta 1996). HSP60 has also been found on the surface of cell membrane in normal cells where it is involved in membrane transport and signalling (Soltys and Gupta 1997). Recent studies show the secretion of HSP60 in extracellular milieu by microglia upon encounter with stress stimuli such as exposure to pro-inflammatory cytokine IL-1β (Swaroop et al. 2016).

HSP60 majorly functions as the mitochondrial chaperone and assists in the process of protein folding in mitochondria (Ostermann et al. 1989). It also stimulates folding of proteins through forced unfolding (Lin et al. 2008). HSP60 can also be expressed on the extracellular surface of stressed cells, acting as a danger signal to the immune system. Apoptotic tumour cells often express HSP60 on cell surface and cause dendritic cell activation which helps in mounting of antitumor T-cell responses against the tumour (Feng et al. 2002).

HSPE family works in close association with HSPD family. It consists of a single member—HSPE1/HSP10—which is also known as GroES. GroES acts as a co-chaperone along with HSPD1/HSP60/GroES. It caps the ATP-activated HSPD1/GroEL on the top of the apical domain and assists HSPD/GroEL in protein folding (Weissman et al. 1995).

Apart from HSP60, the members of other HSP families also serve a variety of cellular functions like protein folding, degrading misfolded proteins, protein refolding, etc. Few of the HSPs have a significant role to play in the context of neuroprotection. For example, HSP90 counteracts neuronal protein aggregation in neurodegenerative disease like Alzheimer's and Parkinson's (Kakimura et al. 2002;

Table 1 Brief summary of heat shock protein family on the basis of their location, function and expression

Gene family	Name	Cellular location	Main function	Expression	References
HSPA family (HSP70s)	– HSPA1A/HSP72 – HSPA2/HSP70-2 – HSPA6/HSP70B′ – HSPA8/Hsc70	Primarily cytosolic	Assembling and folding of proteins, preventing aggregation of unfolded proteins, refolding of denatured proteins	Ubiquitous	Daugaard et al. (2007), Ni et al. (2009), Ran et al. (2000), Shiber and Ravid (2014)
	– HSPA5/Grp78/Bip	Endoplasmic reticulum			
	– HSPA9/Grp75/mortalin	Mitochondria			
HSPC family (HSP90)	– HSPC1/HSP90α	Cytoplasm	Binds to proteins, prevents aggregation of unfolded proteins, degradation of unfolded proteins	Ubiquitous	Sreedhar et al. (2004), Csermely et al. (1998), Masgras et al. (2017), Yang and Li (2005)
	– HSPC3/HSP90β	ER			
	– HSPC4/GPR94 – HSPC5/TRAP1	Mitochondrial matrix			
DNA family (HSP40)	DNAJA (type 1) – DNAJA2/Rdj2 – DNAJA3/TiD1	Postsynaptic membrane	Helps HSP70 in protein folding	Ubiquitous muscle (endplate)	Qiu et al. (2006), Caplan et al. (1993)
	DNAJB (type 2) – DNAJB2/Hsf1 – DNAJB6/Mrj – DNAJB1/Hdj-1	Cytosol, cytoplasmic face of ER		Ubiquitous	
	DNAJC (type 3) – DNAJC5/CSP-α – DNAJC6/auxilin – DNAJC13/RME-8 – DNAJC26/GAK	Cytosol (synapse)			
HSPH family	HSPH1/HSP105	α-cytosol β-nucleus	Functions as nucleotide exchange factor for HSPA family	High in the brain	Saito et al. (2007), Satoh et al. (1998)

HSPB family (small HSPs)	– HSPB1 (HSP27/ HSP25) – HSPB5 (αβ crystallin)	Cytoplasm/nucleus	Chaperone activity, stabilization of cytoskeleton	Glia	Quraishe et al. (2008), van den IJssel et al. (2003)
	– HSPB6 (HSP20)	Cytoplasm			
	– HSPB8 (HSP22)				
HSPD family	HSPD1/HSP60	Mitochondrial matrix	Mitochondria-protein folding Cytosol—control of signal transduction, apoptosis, glycolysis	Astrocytes, neurons, microglia, oligodendrocytes, ependymal cells	Gupta and Knowlton (2002), Cechetto et al. (2000), Pfister et al. (2005), Soltys and Gupta (1996), Martin et al. (1991)
		Extramitochondrial site—cytosol, surface of non-neuronal cells, extracellular space	Cell membrane—membrane transport, cell-cell signalling, immune system alerting Intercellular interstitium—either pro or anti-inflammatory		
HSPE family	HSPE1/HSP10	Mitochondrial matrix	Acts as co-chaperone for HSPD1 family	Ubiquitous	Weissman et al. (1995)
Non-mammalian heat shock family	HSP100/mtHSP78/ HSP104	Mitochondria	Has role in protein quality control by solubilizing aggregated proteins, restoring them to their native conformation	Yeast	Doyle and Wickner (2009), Lo Bianco et al. (2008)

Falsone et al. 2009). Genetic studies done in the recent past show the involvement of DNAJ/HSP40 chaperone in pathogenesis of Parkinson's disease (Appel-Cresswell et al. 2014; Lorenzo-Betancor et al. 2015). Studies have also shown that mutations in HSPB1, HSPB3 and HSPB8 are associated with development of hereditary peripheral neuropathies (Mandich et al. 2010; Vicart et al. 1998). Pathogenesis of neuropathies like Charcot-Marie-Tooth disease (CMT) and distal hereditary motor neuropathy (dHMN) have been associated with mutations in HSPB1 and HSPB8 genes in an Italian cohort (Capponi et al. 2011).

3 Cellular Functions of HSPs

3.1 Chaperone Activity

HSPs act as molecular chaperones and bind to nascent polypeptide chains, assist them in proper folding and prevent the aggregation of misfolded proteins and degradation of improperly folded proteins. HSP60 plays a salient role in importing proteins into mitochondria. It mediates ATP-dependent folding of mitochondrial resident proteins, prevents incorrect folding and also assists in exporting of proteins from mitochondria (Koll et al. 1992). HSP90 prevents unfolded proteins from aggregating and promotes protein folding without ATP usage, in spite of possessing an ATPase domain (Picard 2002). sHSPs can prevent irreversible aggregation of protein in stress conditions (Jakob et al. 1993).

3.2 Modulators of Apoptosis

HSPs play a key role in apoptosis regulation. These include various anti-apoptotic and pro-apoptotic proteins that interact with other cellular proteins, for example, Hsp27 behaves as an anti-apoptotic protein and can inhibit cytochrome-c-mediated activation of caspases in cytosol, it prevents apoptosome formation and thus negatively regulates apoptosis (Bruey et al. 2000). HSP70 can prevent apoptosis by inhibiting Apaf-1 function (Saleh et al. 2000). HSP60 and HSP10 are present in a complex with procaspase-3 in mitochondria. These chaperones make procaspase-3 more prone to cytochrome-c activation and play a role in amplification of caspase cascade (Samali et al. 1999).

3.3 Interaction with Cytoskeletal Proteins

HSPs play a crucial role in fine-tuning the functioning of various cytoskeletal elements as and when required. Upon exposure to stressful situations, cells often respond by modulating their cytoskeleton and increasing the synthesis of HSPs. Few small HSPs act as actin-binding protein and interact with the actin cytoskeleton. For example, previous experiments by Geiger's group have shown that HSP25 from

avian gizzard is capable of preventing actin polymerization by blocking the barbed end of the growing filament (Miron et al. 1988).

3.4 Regulatory Role in Neurodevelopment

Development of embryonic and postnatal stages of multiple organ systems, including nervous system under normal physiological conditions, is promoted by HSPs. Work done on mouse brain by Loones and group shows the close association of HSPB1 with cortical neurons and radial glia that undergoes differentiation highlighting the expression of heat shock protein during embryonic neurodevelopment (Loones et al. 2000). HSPs also play a fundamental role in guiding neuronal and glial differentiation (Cheng et al. 2016; D'Souza and Brown 1998).

4 Role of HSPs in Health and Disease

4.1 Neuroprotection

HSPs are neuroprotective when it comes to many pathological conditions of the brain. They play an integral role in protein folding, removal of misfolded proteins and striking a balance between aggregation and degradation of proteins in a cell such that excess of unfolded proteins does not lead to neurotoxicity. For example, transgenic overexpression of HSPA1/HSPB1 in mouse models of Alzheimer's disease brings down Aβ plaque formation and cognitive dysfunction, having an overall neuroprotective effect by acting as a buffer to the progression of disease (Hoshino et al. 2011; Tóth et al. 2013).

HSPs affect neuronal survival, inflammation and signalling processes also. Exogenous addition of HSPs has an overall anti-inflammatory effect in different acute brain injuries, for example, addition of HSPA8 prevents axotomy-induced death of sensory neurons and is thus useful in repair of peripheral sensory nerve damage (Houenou et al. 1996).

4.2 Cancer

HSPs play a significant role in different molecular mechanisms that lead to carcinogenesis. Various HSPs have been characterized in playing their part in metastasis, angiogenesis, cell proliferation and developing resistance to cancer drugs. Quite a few HSPs have also been characterized as biomarkers in tumour progression (Glaessgen et al. 2008; Melle et al. 2006). For instance, TiD1 belongs to HSP40 family and acts as a tumour suppressor in head and neck squamous cell carcinoma (HNSCC) (Chen et al. 2018).

Angiogenesis is the growth of blood vessels around a tumour to ensure continuous blood supply to the tumour for its growth. HSP27 is found in human tumour microenvironment and stimulates the transcription of vascular growth endothelial factor (VEGF) which leads to tumour growth and angiogenesis (Thuringer et al. 2013).

Many HSP inhibitors are currently in the pipelines that have shown potential in antitumour activity against cancer cells in animal models. AUY922 is a HSP90 inhibitor which is currently undergoing phase-2 clinical trials for patients with advanced non-small cell lung cancer (Felip et al. 2018).

4.3 Diabetes

Expression of heat shock proteins is markedly altered in insulin-resistant and diabetic patients. In a study done by Tiss and group, obese subjects with diabetes showed a decline in the expression and secretion of HSP60 along with an increase in the expression levels of inflammatory and glycaemic markers as compared to the non-diabetic group. This decrease in HSP60 expression was reverted by physical exercise, showing the beneficial effects of physical exercise in improving heat shock response in diabetes (Khadir et al. 2018).

5 HSP60: Warrior in Neuroinflammatory Battles

In addition to their chaperone activities and other cellular functions, HSPs act as potent intercellular signalling molecules that serve as danger signals to the innate immune system and help in immune functions (Chen et al. 1999; Wallin et al. 2002). It has been shown that immune responses directed against members of the HSP60, HSP70 and HSP90 families are implicated to contribute to the pathogenesis of a variety of tissue-specific autoimmune disorders, vascular diseases and inflammatory skin disorders (Bayramgürler et al. 2004; Imamura et al. 2005; van Eden et al. 2005). In particular, HSP60 has been described as immunomodulator molecule in various diseases and manifests a potential immunoregulatory role in the development of autoimmune disorders such as rheumatoid arthritis, type 1 diabetes and in vascular diseases like arteriosclerosis (Abulafia-Lapid et al. 1999; Kamphuis et al. 2005). It has been reported to act as a pathogenic protein as well as to induce immunity in various bacterial and viral infections (Mayr et al. 1999; Noll and AutenriethIb 1996; Yang et al. 2014). HSP60 mediates neuron-glia interaction as well. However, scarcity of research investigating HSP60's role in microglial activation and neuroinflammation makes it a compelling molecular candidate in present-day research. It is much needed to unwind HSP60's functional role and signalling pathways to develop effective therapeutic targets for neuroinflammatory and neurodegenerative conditions.

6 Structure of HSP60

Most of the information about HSP60 has been derived from studies based on GroEL, which is the *Escherichia coli* form of HSPD1/HSP60. GroEL comprises of 14 identical subunits arranged back-to-back in the form of two heptameric rings (Saibil et al. 1993). GroEL forms a barrel-kind of structure with a central channel where protein folding takes place. GroEL has three domains which are as follows:

1. **Equatorial domain**—It is the largest domain. It consists of two subdomains—E1 and E2. The equatorial domains comprise of α-helical sections, which mediate interactions between the two heptameric rings. It also contains the ATP binding site (Ranson et al. 2001).
2. **Intermediate domain**—It connects the equatorial and the apical domains and is composed of two segments—I1 and I2. This domain houses catalytic residues important for ATP hydrolysis (Braig et al. 1994).
3. **Apical domain**—It is made up of a single segment that connects the intermediate domain (Xu et al. 1997). It acts as substrate binding site and also binds to HSPE1/GroES (Fenton et al. 1994; Chen et al. 1994).

GroES is the *E. coli* form of HSP10, and it acts as the co-chaperone. It exists as a heptamer and binds to GroEL forming a cap.

Figure 1 represents the space-filling model of GroEL-GroES complex, where GroEL is depicted as a barrel-shaped structure with the three domains—apical (red), intermediate (green) and equatorial (blue). GroES is binding on top of GroEL like a cap.

Mechanism of GroEL-GroES Function

GroEL has the misfolded substrate protein bound to its apical domain, which causes binding of seven ATP molecules to it. This ATP binding causes further binding of GroES to GroEL. The misfolded protein now proceeds towards the central channel where it is properly folded. ATP is hydrolysed in cis ring which ensures proper folding of protein. This is followed by further binding of ATP to trans ring, resulting in dissociation of the GroEL-GroES complex and subsequent release of folded protein (Sigler and Horwich 1995; Weissman et al. 1994).

7 Many Faces of HSP60: In Context of Pathological Conditions

HSP60 plays a considerable role in the pathogenesis of many diseases. Work done recently by Swaroop et al. in 2018 highlights the significant increase of HSP60 transcript levels in different diseased brain sections compared to their controls (Swaroop et al. 2018). Brain samples of various non-infectious diseases such as glioma, Parkinson's disease, Alzheimer's disease and stroke showed an increased expression of HSP60 mRNA compared to the controls. Similarly, amongst infectious diseases, brain sections of patients with rabies, cerebral malaria, toxoplasma

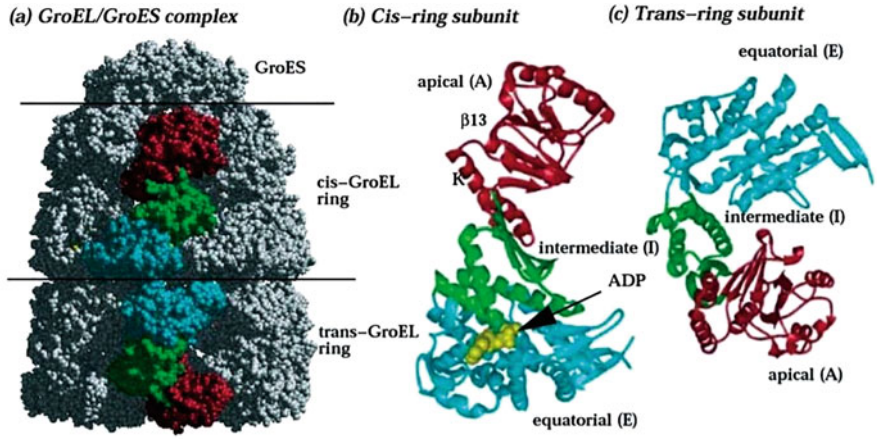

Fig. 1 Space-filling model of GroEL-GroES structure obtained by X-ray crystallography. (**a**) Crystal structure of GroEL/GroES complex, where GroEL is composed of two heptameric rings—cis-GroEL ring and trans-GroEL ring consisting of three domains: apical, intermediate and equatorial, respectively. GroES seen sitting on top of GroEL. (**b**) GroEL assumes cis conformation when ADP is bound to equatorial domain and (**c**) trans-conformation of GroEL in absence of ADP. GroES binds to the top of GroEL as a cap. (Figure adapted with permission from Keskin et al. 2002, Biochemistry. Copyright (2002) American Chemical Society)

encephalitis and cryptococcus meningitis showed a significant increase in mRNA of HSP60 compared to controls.

Research by Lehnardt's group suggests the release of HSP60 by neurons during brain injury in a mouse stroke model (Rosenberger et al. 2015). Glioblastoma patients also express higher amounts of HSP60, which upon being silenced suppresses the progression of the disease (Tang et al. 2016). Expression of HSP60 was upregulated in a microarray analysis of peripheral blood mononuclear cells post *Plasmodium falciparum* infection (Hu 2016).

Following is a detailed account of the different kind of infectious, non-infectious and neurodegenerative diseases in which HSP60 has a key role to play.

7.1 Role in Bacterial Infection

1. ***Listeria monocytogenes***—Functional HSP60 complex is usually expressed in eukaryotic mitochondria, whereas studies in past have also identified HSP60 in subcellular compartments other than mitochondria in conditions of stress or disease (Brudzynski et al. 1992). In one such example, mice infected with intracellular bacterium *Listeria monocytogenes* resulted in increased localization of HSP60 on the plasma membrane of liver and spleen cells compared to the non-infected ones. Increased membrane localization of HSP60 in infected mice is simply an indication that cells are stressed, activated or damaged and are further

targeted for clearance by cytotoxic cells or macrophages as a part of tissue repair process, thus modulating the immune activity of the host (Belles et al. 1999).

2. ***Streptococcus pneumoniae***—Research done by Bansal's group emphasizes the role of HSP60 of *Streptococcus pneumoniae* in inducing protective immunity against a lethal challenge in mice. They observed that GroEL protein (HSP60) of *S. pneumoniae* is immunogenic as it produces high antibody titres in mice but fails to produce IFN-γ. Hence, vaccination with GroEL in mice against a lethal strain of *S. pneumoniae* infection delays the onset of death in them by limiting bacterial growth, but it fails to provide protective immunity against the lethal *Pneumococcus* as IFN-γ is essential for effective clearance of *S. pneumoniae* (Khan et al. 2009).
3. ***Pseudomonas aeruginosa***—*Pseudomonas aeruginosa* is an opportunistic bacterial pathogen responsible for causing respiratory illness. In a study done by Ha's group, human monocytes were infected by *P. aeruginosa* GroEL (bacterial homolog of HSP60) leading to the expression of PTX3 in monocytes (Shin et al. 2017). PTX3 is a pattern recognition receptor of innate immune response, produced by cells in response to inflammatory cytokines which act as stimuli (Bottazzi et al. 2010). GroEL of *P. aeruginosa* acts as a pathogen-associated molecular pattern (PAMP) and has the potential to stimulate expression of PTX3 in human monocytes by utilizing TLR4 and NF-κB signalling pathways. GroEL binds to TLR4, resulting in phosphorylation of IκBα and freeing of NF-κB transcription factors which move to nucleus and increase PTX3 expression. PTX3 plays a crucial role in preventing bacterial infection in the lungs by acting as an opsonin and promoting the phagocytosis of *P. aeruginosa*. Thus, HSP60 has the potential to mount innate immune responses against critically important bacterial pathogens by modulating production of PTX3.

7.2 Role in Viral Infections

1. **Hepatitis B Virus Infection (HBV)**—HBV causes chronic and acute hepatitis in patients, which is a chronic life-threatening liver infection. Earlier studies demonstrated interaction of HSP60 with human HBV polymerase for its maturation into an active state. Studies by Park et al. (2002) depict that HBV Pol requires two minimal sites for its binding with HSP60. By mapping several deletion mutants of HBV Pol, it was observed that TP and RH domains of HBV Pol are necessary for its binding to HSP60 and causing its subsequent activation.
   HSP60 interacts with viral proteins like HBVx protein (HBx) and influences the course of HBV infection. The interaction between HBx and mitochondrial HSP60 promotes apoptosis of infected cells (Tanaka et al. 2004). The viral infection promotes secretion of HSP60 from infected cells, which leads to B cell proliferation. B cells produce IL-10, which decreases production of pro-inflammatory cytokines leading to an overall immunosuppressive effect in HBV patients. HSP60 also causes secretion of IL-10 by regulatory T lymphocytes (Tregs) promoting virus replication and the persistence of HBV infection (Barboza et al. 2007).

2. **Human Immunodeficiency Virus Infection (HIV)**—HIV causes acquired immunodeficiency syndrome (AIDS) which is a potentially life-threatening condition that affects host immunity. HSP60 of the host interacts with gp41, which is one of the key viral proteins of HIV and influences the course of infection. Binding of HSP60 with viral gp41 modulates the antigenicity of gp41 and can block some of the gp41 epitopes, helping the virus escape the immune system (Speth et al. 1999).
   According to a study by Suhrbier's group, circulating HSP60 levels of HIV-infected patients are elevated. This increase in HSP60 possibly contributes to inflammation, immune dysfunction and other non-AIDS clinical events like cardiovascular disease and osteoporosis in HIV patients (Anraku et al. 2012). The same group has also reported a significant reduction in the levels of circulating HSP60 in HIV patients upon combination antiretroviral therapy which might be caused by apoptosis mediated by HSP60 binding to TLR4.
3. **Influenza A Virus Infection**—HSP60 is crucial for influenza A virus replication. The chaperone interacts with polymerase basic protein 2 (PB2) of the viral RNA polymerase and inducts it to the mitochondria. PB2 inside the mitochondria affects the mitochondrial antiviral signalling protein (MAVS), leading to a decrease in interferon-beta (IFN-β) production and thus helps the virus to evade the antiviral host response (Graef et al. 2010). MAVS is a novel protein which mediates activation of transcription factors NF-κB and IRF-3 in response to viral infection, and these transcription factors regulate expression of type-I interferons such as IFN-β which boost antiviral immunity. Thus, when MAVS is affected, as in case with influenza infection, an increase in viral replication is permitted (Seth et al. 2005).

7.3 Role as a Pathogenic Protein

Helicobacter pylori, gram-negative bacteria of mammalian stomach, cause chronic inflammation and gastritis in humans. HSP60 of *H. pylori* acts as a pathogenic protein and leads to the production of interleukin-6 (IL-6) by macrophages of gastric mucosa. IL-6 is an important player in mounting the host defence mechanism and thus controlling the spread of bacteria in vivo. This HSP60-induced IL-6 production by macrophages occurs in a TLR2-, TLR4- and MyD88-independent manner but causes NF-κB activation. Increase in IL-6 levels in the stomach is further correlated with inflammation and gradual progression to gastric cancer (Gobert et al. 2004).

7.4 Role in Neurodegenerative Disorders

1. **Parkinson's Disease**
   Parkinson's disease (PD) involves degeneration of dopaminergic neurons of substantia nigra and neuronal terminal in striatum. Substantia nigra is a part of basal ganglia that controls motor movement in humans. The loss of dopaminergic

neurons of these areas affects movement of the patient. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is often used to induce PD in mice and is widely studied as the MPTP mouse model of PD. Studies by Noelker et al. (2014) show the upregulation of HSP60 mRNA in the mesencephalon and striatum of mice treated with MPTP as well as PD patients.

Studies done on the rat model of Parkinson's disease by Feng et al. (2013) suggest the release of HSP60 by degenerated neurons which activates microglia. Activated microglia release pro-inflammatory cytokines which further cause destruction of dopaminergic neurons. This paves the way for designing of therapeutic strategies to slow down the progression of disease by either preventing the release of HSP60 or interfering with the interaction between HSP60 and microglia.

2. **Alzheimer's Disease**
   Alzheimer's disease (AD) is one of the foremost causes of dementia all over the globe. It is associated with neurotoxic plaque accumulation in the brain. Overexpression of HSP60 alone reduces the cytotoxicity imposed by Aβ plaques to the neurons, suggesting its protective role in Alzheimer's disease (Veereshwarayya et al. 2006). Various in vitro experiments suggest the inhibition of Aβ aggregation by HSP60 involves a more complex mechanism. According to Taglialatela's group, mitochondrial chaperone HSP60 has protective action against Aβ neurotoxicity by changing the toxic Aβ conformation (Marino et al. 2019). Aβ interacts with synapses directly, leading to their degeneration which leads to dementia associated with AD. Treatment of Aβ with HSP60 makes it somewhat lesser synaptotoxic, by decreasing its ability to bind to synapses. HSP60 induces a conformational change in Aβ peptide and inhibits the amyloid fibrillation process. This impairs any further aggregation and subsequently impedes plaque formation (Vilasi et al. 2019). Thus, a deeper understanding regarding HSP60-mediated inhibition of Aβ aggregation is needed for developing treatment strategies against Alzheimer's disease and other such neurodegenerative disorders.
3. **MitCHAP-60 Disease**
   A missense mutation in the HSPD1 gene, which codes for the mitochondrial HSP60 chaperone, has been linked to an autosomal-recessive hypomyelinating leukodystrophy, also known as MitCHAP-60 disease. HSP60 mediates folding of many proteins in mitochondria, and a D3G mutation of mature mitochondrial HSP60 protein leads to its destabilization and impairs its protein folding ability causing the MitCHAP-60 disease (Parnas et al. 2009). This severe neurodegenerative disease affects proper myelin formation and is characterized by profound mental retardation, severe motor impairment, rotatory nystagmus and spastic paraplegia (Magen et al. 2008). Mitochondrial HSP60 thus plays a fundamental role in the process of normal brain myelination, and the lack of it leads to pathogenesis of hypomyelinating neurodegenerative diseases as MitCHAP-60.
4. **Huntington's Disease**
   Huntington's disease is characterized by the development of uncontrolled, irregular and jerky muscle movements in patients. It is progressive in nature and

affects the cognitive and functional abilities of the patient. The disease is marked with an expansion of polyQ repeats at the NH_2 terminus of the huntingtin protein (htt); hence it is also known as polyglutamine (polyQ) disease. This expansion creates an accumulation of mutant htt (mhtt) protein in the form of detergent-insoluble aggregates which are neurotoxic. Studies by Zhu and his group showed that overexpression of ATP synthase α in a polyQ disease model led to removal of mutant htt protein and a subsequent reduction in the formation of inclusion bodies (Wang et al. 2009). ATP synthase α is a part of the F0-F1-ATP synthase which belongs to the HSP60 family of stress proteins. Thus, ATP synthase α-based suppression of polyQ inclusions depicts the neuroprotective role of ATP synthase α which is essentially a HSP60 protein in preventing the progression of Huntington's disease.

7.5 Role as an Immunomodulator

HSP60 acts as a key molecule in intercellular immune networks, by interacting with both the innate and adaptive arms of immunity in mammals (Chen et al. 1999; Wallin et al. 2002; Abulafia-Lapid et al. 1999). HSP60 modulates the innate immune system by activating TLR2 and TLR4 (Vabulas et al. 2001). Depending on the HSP60 accumulation in the milieu, pro-inflammatory or anti-inflammatory responses are mounted.

HSP60 can activate innate immune cells such as monocytes by TLR4 signalling which upon activation produces pro-inflammatory molecules (Chen et al. 1999). Cohen and colleagues reported that human HSP60 induced naive mouse B cells to proliferate and secrete IL-10 and IL-6. These HSP60-treated B cells could further activate an allogenic T-cell response and enhance the secretion of IL-10 and IFN-γ by the responding T cells (Cohen-Sfady et al. 2005). In another study by the same group, HSP60 was shown to inhibit apoptosis of B cells, induced by dexamethasone. This inhibition of B-cell apoptosis is associated with an upregulation of anti-apoptotic molecules—Bcl-2, Bcl-X_L, etc. (Cohen-Sfady et al. 2009).

HSP60 in humans induces gene expression of T_h1 promoting cytokines IL-12 and IL-15 (Flohé et al. 2003). In vitro experiments by Zanin-Zhorov et al. (2005) suggest the role of HSP60 in the differentially modulated expression of T_h1/T_h2 transcription factors in human T cells. HSP60 downregulates the expression of T-bet, NF-κβ and NFATp and upregulates GATA-3 expression.

8 Microglia and HSP60: Partners in Crime

Neuroinflammation is the innate immune response of the central nervous system that is generated by glial cells following immunological insults or neuronal damage. A set of complex immune reactions are mounted by the immune cells to neutralize the invader and restore homeostasis of the tissue. Microglia are the sentinels of immune system of the brain that is involved in neuroinflammation. When facing any danger,

activation of microglia occurs which leads to subsequent upregulation and secretion of pro-inflammatory cytokines and chemokines to ward off the assault. However, inflammation in CNS is like a double-edged sword. On one hand it safeguards the CNS from insults, whereas on the other hand it accelerates neurodegeneration during exaggerated inflammatory response (Wyss-Coray and Mucke 2002).

Activated microglia produce many heat shock proteins which further modulate its activity. It is essential to understand microglial inflammation in the context of heat shock proteins. HSP60 is a chaperone of utmost importance in IL-1β-induced microglial inflammation (Swaroop et al. 2016).

HSP60 is released as a danger signal by apoptotic and necrotic cells of CNS upon brain injury. Release of HSP60 causes activation of microglia which are the innate immune cells of CNS, by initiating the TLR4-MyD88 signalling pathway. Many neurological disorders are characterized by microglial activation. Activated microglia further release neurotoxic molecules leading to neuronal cell death, loss of oligodendrocytes and axonal injury (Lehnardt et al. 2008). Studies done by Wang's group suggest the release of HSP60 by LPS-activated microglia that binds to oligodendrocyte precursor cells triggering its apoptosis (Li et al. 2017).

Other examples that depict the involvement of HSP60 in microglial activation include paraquat, a herbicide that activates microglia leading to an inflammatory response by enhancing the expression and secretion of pro-inflammatory cytokines like tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) in a HSP60/TLR4 signalling pathway (Sun et al. 2018).

In another study, oxymatrine (OMT), an alkaloid, exerts anti-inflammatory effect on LPS-induced microglia cells by inhibiting the HSP60-TLR4-MyD88-NF-κB pathway. OMT suppressed the levels of iNOS, TNF-α, IL-1β and IL-6, preventing microglial activation and thus imparting a neuroprotective role (Ding et al. 2016b). Curcumin attenuates microglial activation by modulating neuroinflammation by the HSP60/TLR-4/MyD88/NF-κB signalling pathway (Ding et al. 2016a). Thus, it is clearly seen that HSP60 plays a fundamental role in influencing the activation of microglia by regulating inflammation pathways.

A diagrammatic representation of HSP60 which acts as a crosstalk molecule between neuron and microglia has been made for better understanding of readers (Fig. 2). The figure highlights the critical role played by HSP60 upon getting released by degenerated neurons and causes microglial activation by binding to TLR4 on its surface. HSP60-TLR4 binding leads to activation of various inflammation pathways in microglia causing secretion of cytokines and chemokines, establishing an inflammatory milieu that further accentuates neuronal damage.

8.1 Role of Microglia in Neuroinflammation

Microglia upon facing hazardous stimuli get activated which is characterized by extensive proliferation, altered morphology and chemotaxis. Microglia can be broadly categorized into M1 and M2 phenotypes. Resting-state microglia is usually in the M0 state, and M1 is the classically activated microglia that release

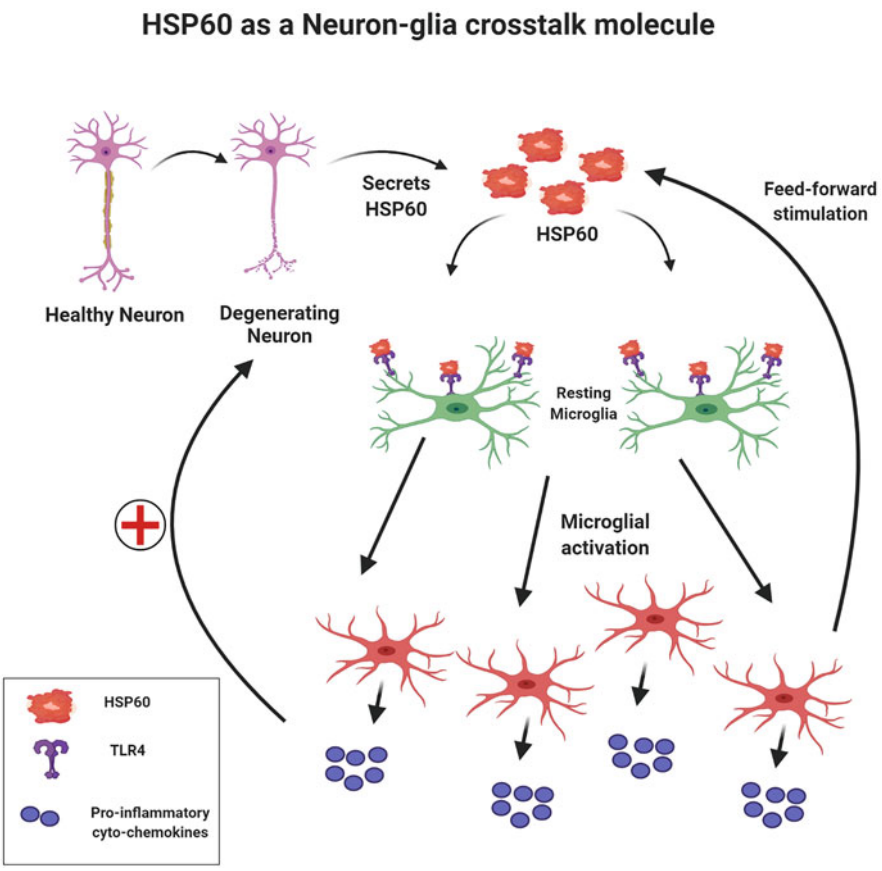

Fig. 2 HSP60 acts as neuron-glia crosstalk molecule. Degenerating neuron secretes HSP60 which activates microglia by binding to TLR4 receptor on its surface and leading to microglial activation. Activated microglia secrete various pro-inflammatory cytokines and chemokines which exacerbate neuronal injury and neurodegeneration. (Created with BioRender.com)

pro-inflammatory mediators which are destructive and promote neuroinflammation. M2 activated microglia usually secrete trophic factors and help in resolving inflammation (Hu et al. 2015). Resting-state microglia have long and slender processes and a more ramified morphology, whereas activated microglia have an amoeboid morphology with less cellular processes. Activated microglia release various pro-inflammatory cytokines like monocyte attractant protein (MCP-1) (Inose et al. 2015), interleukin-1β (IL-1β), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) (Wang et al. 2015), chemokines like inducible nitrogen oxygen synthase (iNOS), cyclooxygenase-2 (COX-2) and reactive nitrogen species (Boje and Arora 1992) to fight infections. Different anti-inflammatory cytokines like transforming growth factor-beta1 (TGF-β1) (Kiefer et al. 1998), interleukin-10

(IL-10) (Jander et al. 1998) and IL-1 receptor antagonist (IL-1Ra) (Liu et al. 1998) are also secreted by microglia.

Reports by Kaushik et al. in 2010 (Kaushik et al. 2010) show an increased expression of pro-inflammatory mediators MCP-1, TNF-α, IL-6, iNOS and COX-2 in BV2 mouse microglial cell line upon lipopolysaccharide (LPS) treatment. LPS is often used to stimulate neuroinflammation; it is a ligand of TLR4 which is present on microglia (Lehnardt et al. 2002). Microglial stimulation by LPS leads to its activation and release of pro-inflammatory cytokines which further mediate neuroinflammation.

Role of IL-1β in Microglial Activation

Interleukin-1β (IL-1β) plays a crucial role in microglial activation. It upregulates numerous stress proteins and pro-inflammatory factors in microglia and facilitates different signalling pathways to induce inflammation; hence it is known as the 'master regulator of inflammation'. Reports by Kaushik et al. (2013) show that microglial stimulation by IL-1β causes production of endogenous IL-1β as well as other pro-inflammatory mediators as COX-2, MCP-1 and IL-6.

IL-1α and IL-1β independently bind to the type-1 receptor (IL-1R1), and another ligand IL-1 receptor antagonist (IL-1RA) binds to IL-1R1 but does not trigger downstream signalling events. IL-1 receptor accessory protein (IL-1RAcP) serves as a co-receptor, necessary for IL-1 signal transduction. Binding of ligand to IL-1R1 facilitates recruitment of IL-1RAcP and then assembles intracellular signalling proteins—myeloid differentiation response gene 88 (MyD88) and tumour necrosis factor associated factor 6 (TRAF6)—leading to activation of different kinases including IL-1 receptor-associated kinase 1 and 4 (IRAK1, IRAK4). IRAK1, IRAK2 and TRAF6 dissociate from initial receptor complex and cause activation of transcription factors—nuclear factor κB (NF-κB) and activator protein 1 (AP-1) transcription factor complex consisting of Fos, Jun and ATF families of transcription factors.

Figure 3 shows the interaction of different IL-1 ligands with IL-1 receptor and the subsequent activation of nuclear factor κB signalling and various MAPK signalling pathways leading to expression of IL-1 target genes.

Previous studies from our lab show that microglial stimulation with IL-1β leads to endogenous IL-1β production as well as expression of mediators as MCP-1, IL-6 and COX-2. The work shows that upon IL-1β stimulation of microglia, Klf-4 is induced which is a transcription factor that regulates production of pro-inflammatory mediators via PI3K/Akt pathway and activates microglia (Kaushik et al. 2013). In order to unravel the underlying mechanism of IL-1β-induced microglial activation and to decipher the role of HSP60 in modulating this mechanism, attempts were made by our group which are described as follows (Swaroop et al. 2016, 2018).

Administration of IL-1β Causes Microglial Activation Inducing Inflammation Both In Vitro and In Vivo

We found a significant increase in expression of pro-inflammatory enzymes—iNOS and COX2—as well as pro-inflammatory cytokines, TNF-α, MCP-1 and IL-6, both

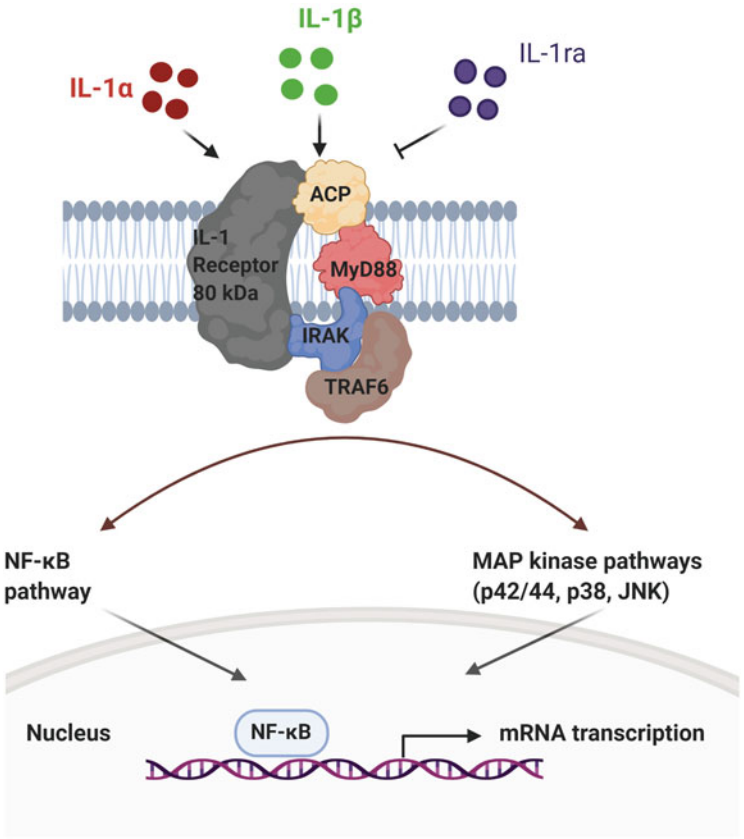

Fig. 3 Mode of action of IL-1β. IL-1α, IL-1β and IL-1ra are the ligands which can bind to IL-1 receptor. IL-1α and IL-1β bind to type I IL-1 receptor (IL-1R1), whereas IL-1ra binds to type II IL-1 receptor (IL-1R2). IL-1RAcP acts as a co-receptor and causes recruitment of adaptor protein MyD88 and IRAK-2 and TRAF6. This complex causes transcription of NF-κB and MAPK genes. (Figure re-prepared from Rothwell and Luheshi, Trends in Neuroscience, 2000) (Created with BioRender.com)

in N9 murine microglial cells (in vitro) and BALB/C mouse brain (in vivo) upon treatment with IL-1β. This further strengthens the role of IL-1β in inducing inflammation in both microglial cells and mouse brain.

Proteomic Profiling Depicts Increased Expression of HSP60 Post-IL-1β Treatment of Microglial Cells

We decided to do proteome analysis of N9 microglial cells in response to IL-1β treatment to have a holistic idea of the different proteins and signalling pathways evoked due to IL-1β-induced inflammation. Proteomic analysis of IL-1β-treated and IL-1β-untreated N9 microglial cells at different time points revealed modulation of 21 proteins.

These spots were processed for MALDI TOF/TOF MS and MS/MS analysis which led to identification of 17 different types of proteins. To further classify the identified proteins functionally, GeneCodis3 software was used. These differentially regulated proteins were functionally classified based on their molecular functions. Few major classes were nucleotide binding, ATP binding, unfolded protein folding, identical protein binding, electron carrier activity, etc. Unfolded protein binding was found to be one of the top-notch molecular functions, and on analysing functional connectivity of the identified proteins using STRING database, HSP60 was found to have the highest number of interactions of all the proteins identified. This motivated our group to investigate the role of HSP60 in inducing microglial activation and inflammation.

IL-1β Administration Leads to Increased Expression of HSP60 Both In Vivo and In Vitro and Aids Its Secretion into Extracellular Milieu

To this end, western blot and quantitative real-time PCR experiments of both N9 microglial cells and mice brain treated with IL-1β for different time points were performed which showed an increased expression of HSP60 at both protein and transcript levels.

Immunostaining experiments also showed the activation of microglial cells upon IL-1β treatment, as seen by increased expression of Iba1 which is a microglial marker. Also, co-localization of both HSP60 and Iba1 in both N9 microglia cells and primary microglia confirmed the increased expression of HSP60 by activated microglia on IL-1β treatment.

HSP60 was previously reported by many groups to be secreted by neurons to activate microglia and other antigen-presenting cells by binding with TLR4 present on the cell surface (Lehnardt et al. 2008; Li et al. 2017; Rosenberger et al. 2015). Therefore, HSP60 levels were assessed in the secretome of microglial cells post-IL-1β treatment by western blotting. The results showed significantly upregulated expression of HSP60 in the extracellular milieu of the IL-1β-treated cells compared to control. This suggests that upon IL-1β treatment of microglia, the increase in expression of HSP60 is not only at the intracellular level, but it also gets secreted out to the extracellular environment.

Before diving deep into the intricacies of HSP60-induced inflammation in microglia, it is imperative to understand the importance of Toll-like receptors which is a pattern recognition receptor and has a fundamental role in establishing an early innate immune response to the invading pathogens.

Pattern Recognition Receptors in Microglia

Pattern recognition receptors (PRR) are specialized receptors that are encoded in germ line and are expressed on various immune cells. Microglia express several PRR involved in ligand-mediated activation of microglia in the CNS (Hanke and Kielian 2011). PRRs recognize conserved molecular structures known as pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) found on microbes. PRRs are broadly divided into five families in mammals:

1. Toll-like receptors (TLRs)
2. Nucleotide-binding oligomerization domain-like receptors (NLRs)
3. C-type lectin receptors (CLRs)
4. RIG-1 like receptors (RLRs)
5. AIM2-like receptors (ALRs)

Stimulation of PRRs on microglia leads to their activation and helps the microglia in presenting the antigen to $CD4^+$ T cells.

Role of TLR4 in HSP60-Induced Inflammation

Toll-Like Receptors and Their Structure

TLR family comprises of 10 members (TLR1–TLR-10) in humans and 12 members (TLR1–TLR9, TLR11–TLR13) in mouse (Kawasaki and Kawai 2014).

TLR is composed of the following:

1. An ectodomain with leucine-rich repeats (LRRs) that mediate PAMP recognition.
2. A transmembrane domain.
3. A cytoplasmic Toll/IL-1 receptor (TIR) domain (Monie et al. 2009).

TLRs interact with PAMPs/DAMPs and then recruit TIR domain-containing adaptor proteins such as MyD88 and TRIF leading to the activation of NF-κBs or MAPs and regulate expression of inflammatory genes.

Work done by Lehnardt et al. in 2008 (Lehnardt et al. 2008) shows that HSP60 serves as a signal of CNS injury by activating microglia through TLR4-MyD88-dependent pathway. We did co-immunoprecipitation experiment to check the interaction of HSP60 secreted by microglia with TLR4 in response to IL-1β treatment. With the increase in levels of HSP60, there was a subsequent rise in TLR4 expression in microglial cells depicting the binding of TLR4 with HSP60.

To decipher the role of TLR4 in IL-1β-induced inflammation, a specific inhibitor—CLI-095 (Invivogen)—was used against TLR4. Inhibiting TLR4 in microglial cells led to the significant reduction in the expression levels of the major pro-inflammatory cytokines—MCP-1, TNF-α and IL-6—and pro-inflammatory enzymes iNOS and COX-2, suggesting the important role of TLR4 in IL-1-β-mediated signalling in microglia.

Our studies revealed that IL-1β activates microglia by binding to its receptor IL-1R1 and enhancing the expression of HSP60 in microglia. HSP60 is secreted out of microglia and acts as a TLR4 ligand, binds to it and leads to activation of the following pathways.

1. **Phosphorylation and nuclear localization of NF-κB causing transcription of NLRP3 gene and pro-IL-1β**
   HSP60 and TLR4 binding facilitates phosphorylation of NF-κB and causes its nuclear localization. NF-κB upon nuclear localization acts as a signal for

activation of NLRP3 inflammasome pathway which in turn leads to endogenous IL-1β production (Swaroop et al. 2018).

2. **Induction of mitochondrial damage by elevating ROS levels**
   HSP60-TLR4 binding can also cause a decrease in mitochondria membrane potential in microglia and generation of mitochondrial ROS indicating the damage of mitochondria. This also leads to NLRP3 inflammasome activation causing cleavage of pro-caspase-1 to caspase-1 which leads to endogenous IL-1β production (Swaroop et al. 2018).
3. **Activation of MAPK pathway**
   Binding of HSP60 with TLR4 in microglia causes a surge in inflammation by phosphorylation of all MAPK pathways. This results in an increased production of pro-inflammatory cytokines which forms a feed-forward loop of inflammation in microglia (Swaroop et al. 2016). Binding of HSP60 to TLR4 can also activate the NLRP3 inflammasome pathway leading to endogenous IL-1β production. NLRP3 inflammasome activation occurs via two ways:
   (a) Phosphorylation and nuclear localization of NF-κB which transcribes NLRP3 gene and pro-IL-1β.
   (b) Induction of mitochondrial damage which leads to ROS generation that also aids in formation of NLRP3 inflammasome.

Formation of NLRP3 inflammasome causes the cleavage of pro-caspase-1 into caspase-1 which is also known as interleukin converting enzyme (ICE). This is the executioner step of inflammasome pathway, which leads to maturation of pro-IL-1β into IL-1β.

8.2 HSP60 Plays a Crucial Role in IL-1β-Mediated NF-κB Phosphorylation and Its Nuclear Localization

NF-κB phosphorylation activates NLRP3 inflammasome pathway which leads to IL-1β production. Work done by Swaroop et al. in 2018 (Swaroop et al. 2018) show that NF-κB phosphorylation is induced by IL-1β treatment, both in N9 microglia cells and BALB/C mouse model. To assess the role of HSP60 in IL-1β-induced NF-κB phosphorylation, it was overexpressed in both the models and then treated with IL-1β, which led to the induction of NF-κB phosphorylation as detected by the increased expression of p65-NF-κB in western blotting. And upon knocking down HSP60, NF-κB phosphorylation was not induced despite IL-1β treatment in both in vitro and in vivo models.

For the proper functioning of NF-κB, it must be translocated into the nucleus on being phosphorylated and only then can it regulate the expression of inflammatory genes. In both the models, overexpression of HSP60 caused an increase in nuclear localization of NF-κB. Phosphorylated p65-NF-κB was localized into the nucleus in both N9 microglia cells and BALB/C mouse brain as detected by western blotting, whereas knockdown of HSP60 in both the models led to a decrease in NF-κB nuclear localization even upon IL-1β treatment.

NF-κB is considered as the classic pro-inflammatory signalling pathway that plays an important role in inflammation and immune response. NF-κB is found in an inactive state in association with IκB in resting cells. Degradation of IκB by proteasome in presence of stimuli causes NF-κB phosphorylation and its translocation into the nucleus (Sen and Smale 2010; Liu et al. 2017). Phosphorylated NF-κB transcribes NLRP3, pro-IL-1β and pro-IL-18 which are necessary for inflammasome activation.

Upon confirming the IL-1β-induced phosphorylation and nuclear localization of NF-κB in a HSP60-dependent manner, we tried to find the role of HSP60 in IL-1-β-induced NLRP3 expression (Swaroop et al. 2018).

8.3 HSP60 Regulates Endogenous IL-1β Production in Activated Microglia by Stimulating NLRP3 Inflammasome Pathway

Nuclear localization of pNF-κB facilitates NLRP3 inflammasome activation by inducing the transcription of NLPR3 gene and pro-IL-1β (Bauernfeind et al. 2009). Swaroop et al. in 2018 showed the overexpression of HSP60 both in vitro and in vivo caused the expression of NLRP3 both at transcript and protein levels in the presence of IL-1β treatment. Similarly, downregulation of HSP60 reduces NLRP3 expression in presence of IL-1β treatment in both the models, depicting the crucial role of HSP60 in IL-1β-induced NLRP3 expression.

NLRP3 Inflammasome: Molecular Complex Regulating Caspase Activation

Structure of NLRP3 Inflammasome

NOD-like receptors (NLRs) are intracellular PRRs that recognize PAMPs and DAMPs. NLRP inflammasomes are multimolecular complexes that are large in size and are involved in caspase-1 activation (Martinon et al. 2002). This protein complex acts as a molecular stage and aids in converting caspase-1 into its active form, further controlling the maturation and secretion of interleukins IL-1β and IL-18. The pro-inflammatory cytokines thus produced are associated with a variety of innate immune processes and instigate inflammation. The basic structure of NLR family comprises of the following:

1. The central nucleotide-binding and oligomerization (NACHT) domain that acts as a cytosolic sensor, flanked by C-terminal leucine-rich repeats (LRRs) and N-terminal pyrin domain (PYD).
2. An adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD). ASC acts as a bipartite molecule consisting of N-terminal pyrin domain and C-terminal caspase activation and recruitment domain (CARD).
3. An effector pro-caspase-1 (von Moltke et al. 2013).

NLRP3 inflammasome is fully characterized and acts as an intracellular receptor comprising of NLRP3 scaffold, the ASC adaptor and caspase-1. NLRP3 is involved in the identification of numerous viruses as varicella zoster virus (Nour et al. 2011), influenza virus (Kanneganti et al. 2006), vesicular stomatitis virus (Rajan et al. 2011), Japanese encephalitis virus and mediating inflammation (Kaushik et al. 2012). Activation of NLRP3 upon virus exposure leads to its oligomerization causing PYD domain clustering and enabling its interaction with the PYD- and CARD-containing adaptor ASC. CARD domain of ASC further recruits CARD of pro-caspase-1 permitting its cleavage into active caspase which converts IL-1β and IL-18 into active forms.

Figure 4 is a pictorial description of NLRP3 inflammasome encompassing its structural domains and functional interaction with pro caspase-1 converting IL-1β and IL-18 into their active forms.

8.4 HSP60 Causes Mitochondrial Damage and Accelerates Production of Reactive Oxygen Species

There are many ways in which NLRP3 inflammasome can be activated, out of which mitochondrial damage leading to reduction of mitochondrial membrane potential and generation of mitochondrial reactive oxygen species (ROS) is a widely studied mechanism. Reports by Zhou et al. in 2011 (Zhou et al. 2011) show the generation of ROS by mitochondria having reduced membrane potential can lead to NLRP3 inflammasome activation.

Swaroop et al. (2018) have shown that in N9 microglial cells, both IL-1β treatment and HSP60 overexpression could expedite mitochondrial damage which was confirmed by the decrease in mitochondrial membrane potential and a significant increase in ROS generation. Mitochondrial membrane potential in microglia was assessed by Rhodamine-123 (Rh 123) assay, whereas ROS generation in mitochondria was detected by DCFDA. However, in the microglial cells with knocked down HSP60, less mitochondrial damage and decreased ROS generation were observed even after IL-1β treatment, suggesting an important role of HSP60 in IL-1β-induced mitochondrial damage involving ROS generation.

HSP60 Plays a Key Role in IL-1β-Induced Caspase-1 Activation

We have already seen the fundamental role of HSP60 in NLRP3 expression in microglia cells and previous knowledge about NLRP3 inflammasome activation playing an active role in caspase-1-mediated conversion of pro-IL-1β to IL-1β. Hence, we next checked the effect of HSP60 overexpression on caspase-1 activation, which is required for IL-1β production. It was observed that both the overexpression of HSP60 and IL-1β in mice model and microglia cells caused an increase in caspase-1 activity, whereas, upon knocking down HSP60 in both these models, a sharp reduction in caspase-1 activity levels was seen despite IL-1β treatment depicting the important role of HSP60 in activation of caspase-1.

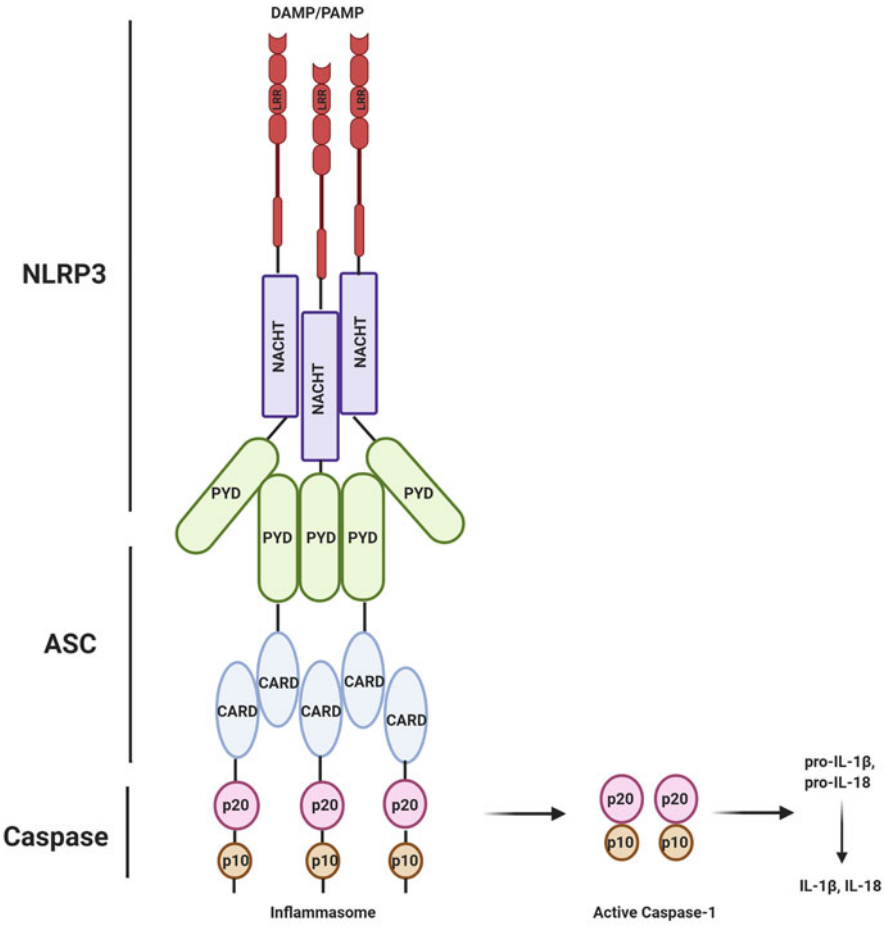

Fig. 4 Model of NLRP3 inflammasome. Comprises of three domains: pattern recognition receptors (PRRs), the adaptor (ASC-CARD) and the caspase. The complex mediates activation of caspase-1 which converts proIL-1β to IL-1β. (Figure re-prepared from Anders and Muruve 2011, Journal of the American Society of Nephrology) (Created with BioRender.com)

8.5 HSP60 as a Mediator of Endogenous IL-1β Production in Activated Microglia

To further understand the role of HSP60 in facilitating endogenous IL-1β production by activated microglia, HSP60 overexpression and knockdown studies were carried out. Both in vitro and in vivo models were used, and the effect of HSP60 on endogenous IL-1β production was checked by qRT-PCR and ELISA experiments. It was observed that IL-1β can upregulate its own production in microglia, which was analysed both in vitro and in vivo at transcript level as well as protein level. Upon overexpressing HSP60 in N9 microglial cells, there was a marked increase in

the endogenous IL-1β production. However, in microglial cells with knocked down HSP60, IL-1β production was reduced in the presence of external stimuli (IL-1β treatment). These results show the pivotal role of HSP60 in microglial IL-1β production both in vitro and in vivo.

It is evident from the above-mentioned observations that HSP60 is released from microglia after IL-1β treatment and binds with TLR4 present on the microglial membrane. This, in turn, induces NLRP3 inflammasome formation and IL-1β production in microglia. Activation of NLRP3 inflammasome involves phosphorylation and nuclear localization of NF-κB, leading to upregulation of NLRP3 and IL-1β gene expression. HSP60-TLR4 binding also plays a regulatory role in inducing mitochondrial stress and ROS generation which triggers NLRP3 inflammasome activation. Inflammasome activation leads to maturation of pro-caspase-1 to caspase-1 in an executioner step which successively enhances the sustained production and secretion of IL-1β. Thus, HSP60 exacerbates neuroinflammation by stimulating endogenous IL-1β production in activated microglia by inducing NLRP3 inflammasome pathway.

Although the mechanism by which endogenous IL-1β production occurs has been elucidated, there remains few missing links. For example, the exact mechanism by which HSP60 induces NF-κB phosphorylation is not known, and also the effect of MAPK in inflammasome activation needs to be uncovered. More investigation is required to unveil the pathway that leads to NF-κB phosphorylation and the subsequent transcription of NLRP3 and pro-IL-1β genes.

8.6 Evaluating HSP60's Contribution in Inducing Microglial Activation and Inflammation

The role of HSP60 in IL-1β production from activated microglia is quite evident from the above signalling mechanism; hence we now tried exploring the role of HSP60 in IL-1β-induced inflammation. To assess the effect of HSP60 on microglial activation, overexpression and knockdown studies of HSP60 were done in N9 microglia cells. Upon overexpressing HSP60 in microglial cells by using mouse HSP60 cDNA clone, the expression levels of pro-inflammatory cytokines—MCP-1, TNF-α and IL-6—and pro-inflammatory enzymes iNOS and COX-2 increased significantly as detected by CBA and western blotting, respectively. This increase in expression of pro-inflammatory mediators was present even in absence of IL-1β treatment. Knocking down HSP60 using an esiRNA led to the decrease in expression levels of aforementioned cytokines to a significant extent in IL-1β-treated microglial cells suggesting the modulatory role of HSP60 in microglial activation leading to inflammation.

8.7 HSP60 Aggravates IL-1β-Induced Microglial Activation and Leads to Inflammation by Phosphorylating Mitogen-Activated Protein Kinase (MAPK)

There have been previous reports of IL-1β-induced activation of MAPK pathways in addition to phosphorylation of NF-κB in the context of inflammation (Wang et al. 2005; Lin et al. 2005). Swaroop et al. in 2016 (Swaroop et al. 2016) demonstrated the role of HSP60 as an antigenic protein and how it activates microglia and induces inflammation by phosphorylating MAPK proteins. Upon knocking down HSP60 in microglial cells, there was a significant decrease in the phosphorylated forms of ERK1/2, JNK and p38 (all belong to MAPK family), and overexpressing HSP60 in microglial cells led to a significant increase in phosphorylation of the preceding MAPKs as detected by western blotting. Before embarking on the mechanism behind IL-1β-induced inflammation assisted by MAPK phosphorylation and HSP60, here are some glances of distinct molecular alleys of inflammation.

Mitogen-activated protein kinase (MAPK) signalling pathway constitutes of serine/threonine kinases that are activated by inflammatory factors, neurotransmitters, stress conditions, viruses, etc. leading to intracellular responses (Cargnello and Roux 2011; Kyriakis and Avruch 2012). MAPK signalling pathway controls essential cellular processes like growth, metabolism and apoptosis. Dysregulation of MAPK pathways can lead to a plethora of diseases like Alzheimer's disease, cancer and multiple sclerosis (Culbert et al. 2006; Krementsov et al. 2013).

MAPK pathways are well characterized in mammals and are classified into three families:

1. Extracellular signal-regulated kinase 1/2 (ERK 1/2)
2. c-Jun amino terminal kinase (JNK)
3. p38 MAPK

Figure 5 shows the three main signalling pathways of MAPK superfamily. MAPK module operates in a three-tier manner—where MAPK is activated by MAPK kinase (MAPKK) which is a dual kinase. MAPKK in turn is activated by MAPKK kinase (MAPKKK) which receives signals from receptor stimulated by signals.

ERK Pathway

It belongs to the family of mitogen-activated protein kinases signalling molecules. Extracellular signal-regulated kinase (ERK) plays diverse roles like proliferation, survival, metabolism and differentiation in cell. This pathway is activated by small G proteins such as Ras, which phosphorylates MEK1/2. MEK1/2 is found upstream of ERK1/2 and phosphorylates it. Downstream molecules of ERK1/2 include kinase suppressor of Ras (KSR), which has various targets inside the cell. Implication of

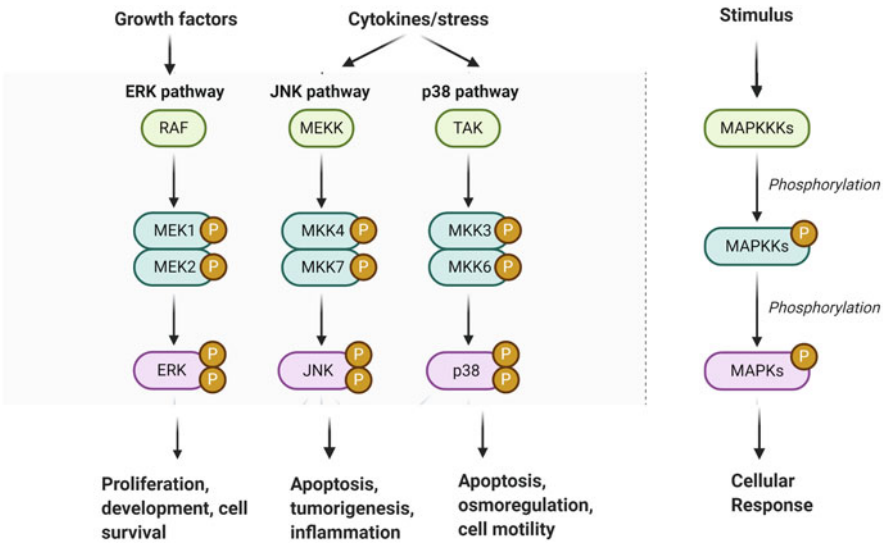

Fig. 5 Brief summary of the three MAPK pathways. ERKs, JNKs and p38 and their upstream kinases—MAPKKK, MAPKK and MAPK—are shown. MAPKKK is activated by numerous signals like growth factors or stressful conditions and triggers activity of MAPKK and MAPK. MAPK upon activation causes transcription of genes associated with development, proliferation, apoptosis and other such cellular activities. (Figure re-prepared from Cowan and Storey 2003, Journal of Experimental Biology) (Created with BioRender.com)

ERK1/2 signalling in several inflammation-related diseases has also been reported (Lu and Malemud 2019; Shaul and Seger 2007).

JNK Pathway

Jun-N-terminal kinase (JNK) is primarily a stress-activated protein kinase that is induced by various environmental as well as intrinsic stressors like ROS, cytokines, UV rays, etc. JNK pathway is associated with diverse cellular functions like proliferation, apoptosis and repair machinery. MAP3Ks are activated by stressors which phosphorylate downstream MAP2K molecules like MKK4 and MKK7 that phosphorylate JNK. Targets of JNK include transcription factor activator protein-1 (AP-1) (Weston and Davis 2007).

p38 MAPK Pathway

p38 MAPK pathway is involved in the biosynthesis of pro-inflammatory cytokines. It is activated in response to cellular stresses and inflammatory cytokines. p38 is one of the kinase pathways that regulates the production of IL-1β and TNF-α. Generally, p38 MAPKs integrate extracellular stimuli through a series of intracellular signalling complexes. p38 MAPK is activated upon being phosphorylated by an upstream

kinase MEK3/6. Activated p38 MAPK then phosphorylates downstream molecules and increases production of pro-inflammatory cytokines (Obata et al. 2000).

8.8 HSP60-Induced Microglial Activation and Subsequent Inflammation Are Dependent on p38 MAPK Pathway

In order to delineate the specific MAPK pathway involved in HSP60-mediated inflammation, specific inhibitors were used for JNK, ERK and p38 MAPK by us. Blocking of p38 MAPK pathway by its inhibitor (SB203580) in the presence of HSP60 showed a marked decrease in expression of pro-inflammatory cytokines—MCP-1, TNF-α and IL-6—and chemokines iNOS and COX-2, whereas blocking of ERK and JNK with their inhibitors—U0126 and SP600125, respectively—did not cause a marked decrease in cytokine and chemokine secretion exhibiting the insignificant role of ERK and JNK in HSP60-induced microglial activation causing inflammation.

Also, to determine the active role of p38 MAPK in HSP60-mediated microglial activation causing inflammation, mitogen/extracellular signal-regulated kinase 3/6 (MEK3/6) was knocked down. MEK3/6 is an upstream molecule of p38 MAPK pathway, responsible for phosphorylating p38 MAPK. A similar decrease in pro-inflammatory cytokines and pro-inflammatory enzymes was observed upon MEK3/6 knockdown. These results confirmed the involvement of p38 MAPK pathway in HSP60-mediated inflammation.

Summing up, we understand that release of HSP60, which is a mitochondrial chaperone, by IL-1β treatment leads to activation of microglia in a TLR4-MEK3/6-p38 MAPK-dependent manner. It was also learnt that IL-1β treatment not only increased HSP60 expression in microglia but also caused its secretion into the extracellular milieu. After getting secreted, HSP60 binds to TLR4 causing further microglial activation and inflammation by activating p38 MAPK via MEK3/6.

This study helped us in speculating a model in which neuroinflammation led to release of HSP60 which activated innate immunity by binding to TLR4 and in turn increasing the inflammatory response of microglia. However, the pathway by which p38 MAPK induces production of inflammatory cytokines is still not known. According to a report, p38 MAPK might regulate cytokine production by a NF-κB-dependent mechanism (Saha et al. 2007), whereas another study depicts the direct involvement of p38 MAPK in stimulating the production of inflammatory mediators (Guan et al. 1998). Lastly, our study suggests HSP60 as a novel target for developing therapeutic interventions in case of various neuroinflammatory conditions, as it regulates IL-1β-induced microglial activation causing inflammation via the HSP60-TLR4-p38 MAPK axis.

9 Microglia and Japanese Encephalitis Virus: Molecular Interdependence of HSP60 and NLRP3 in Regulating Inflammation Pathways

9.1 Introduction to Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) belongs to the family *Flaviviridae* and causes Japanese encephalitis (JE). It is a neurotropic virus and causes inflammation in the brain after initial infection of peripheral tissues. The virus is transmitted by *Culex tritaeniorhynchus* mosquito (Ghosh and Basu 2009). The central nervous system is the major target of JEV, and the infection is characterized by fever, headache and vomiting leading to high mortality and other neurological sequelae in patients that survive it. JEV is a single-stranded RNA virus and majorly affects children aged between 0 and 15 years (Kant Upadhyay 2013; Kumar et al. 1990).

JEV infection is accompanied with severe neuroinflammation, which in turn contributes to neuronal death and worsens the effect of viral infection by modulating production of other cytokines and chemokines in the brain. Reports from our lab have shown a progressive decline in expression of anti-inflammatory cytokine IL-10 in the experimental model of JEV. Lowering of IL-10 levels following JEV infection is correlated with an increase in levels of pro-inflammatory cytokines IL-1β and TNF-α. Low IL-10 levels also promote bystander killing of neurons, aggravating the infection (Swarup et al. 2007b). Studies have shown that JEV induces neuronal apoptosis in mouse and human neuroblastoma cells. JEV infection causes a changed expression of TNFR-1 in neurons which initiates TRADD-mediated neuronal apoptosis. Neuronal death activates both astrocytes and microglia, and the inflammatory cycle is continued (Swarup et al. 2007a). Infection by JEV leads to increased astrogliosis and induces morphological and functional changes in astrocytes by differentially regulating the expression of ROS, IL-1β, IL-8, IP-10 and MCP-1 (Mishra et al. 2008). JEV infection results in a huge loss of actively dividing neural progenitor cell population in the subventricular zone as confirmed in both in vitro neurosphere and in vivo models. Depletion of neural progenitor pool by JEV causes a dysregulation in neurogenesis in the survivors, marked by the development of cognitive defects in them (Das and Basu 2008).

9.2 Microglia Act as Companion in JEV-Driven Neuroinflammation

Neuroinflammation associated with JEV is primarily due to the activation of glial cells and the release of inflammatory mediators from them along with causing neuronal death. JEV-induced microglial activation leads to morphological changes in microglia, transforming process-bearing microglia to a round-shaped darkly stained structure. JEV infection also causes microgliosis which is an increase in the proliferation rate of microglia, along with elevating the expression of TNF-α, IL-1β, IL-6 and RANTES (Chen et al. 2010).

Work done by Ghoshal et al. in 2007 (Ghoshal et al. 2007) utilized an experimental model of Japanese encephalitis virus infection to understand the role played by microglia. The group showed a robust increase in reactive microglia post infection by performing lectin staining which is a marker for microglial activation. The virus caused a surge in levels of iNOS, COX-2, IL-6, IL-1β, TNF-α and MCP-1 in microglia; the activated microglia thus cause irreversible bystander damage to neurons causing their death.

Mishra and Basu (2008) showed the role of minocycline in abrogation of microglial activation in the brain of JEV-infected mice. Iba-1 staining showed a sharp reduction in the number of activated microglia upon minocycline treatment, along with decrease in expression of pro-inflammatory mediators like MCP-1, TNF-α, IL-6, IL-12 and IFN-γ. Reports by Wani et al. in 2020 (Wani et al. 2020) suggest the neuroprotective role of atorvastatin (ATR) in abrogating microglial activation and reduction in production of pro-inflammatory mediators MCP-1, TNF-α and IL-6 in the subventricular zone (SVZ) of JEV-infected mice.

There have been many in-house studies on microRNAs that regulate microglial activation in JEV infection. MicroRNA-29b plays a pivotal role in elevating the levels of pro-inflammatory cytokines in JEV-infected microglial cells (Thounaojam et al. 2014a). Thounaojam et al. in 2014 (Thounaojam et al. 2014b) showed an increased expression of microRNA-155 (miR-155) in JEV-infected microglia and brain samples. miR-155 regulates inflammatory response during JEV infection by targeting the NF-κB pathway in microglia. Inhibiting miR-155 expression led to an overall reduction in microglial activation and secretion of pro-inflammatory cytokines, thus suggesting a novel strategy in managing JEV-related neuroinflammation. According to a study by Hazra et al. in 2019 (Hazra et al. 2019), microRNA-301a was seen in positively regulating JEV-induced microglial activation. When miR-301a was inhibited, the expression of NKRF (target of miR-301a) was restored, which led to reduction in activation of microglia and neuronal death. Unpublished findings from our lab also indicate microglial activation in the spinal cord sections of JEV-infected mice as detected by Iba-1 staining which is a microglial marker (Bhaskar et al., manuscript in preparation).

9.3 Japanese Encephalitis Virus Evokes Activation of NLRP3 Inflammasome

Studies by Kaushik et al. in 2012 (Kaushik et al. 2012) identified the role of NLRP3 inflammasome-mediated caspase-1 activation and the subsequent IL-1β and IL-18 production in mouse brain and microglia cells in the context of JEV infection. In vitro data suggest that inhibiting caspase-1 activity led to the decrease in expression of IL-1β and IL-18 production, highlighting the fact that caspase-1 is crucial for maturation of IL-1β and IL-18 during JEV infection. NLRP3 inflammasome also stands out as a key mediator of caspase-1 activity and IL-1β and IL-18 production in microglia as knocking it down decreases caspase-1 activity and the subsequent

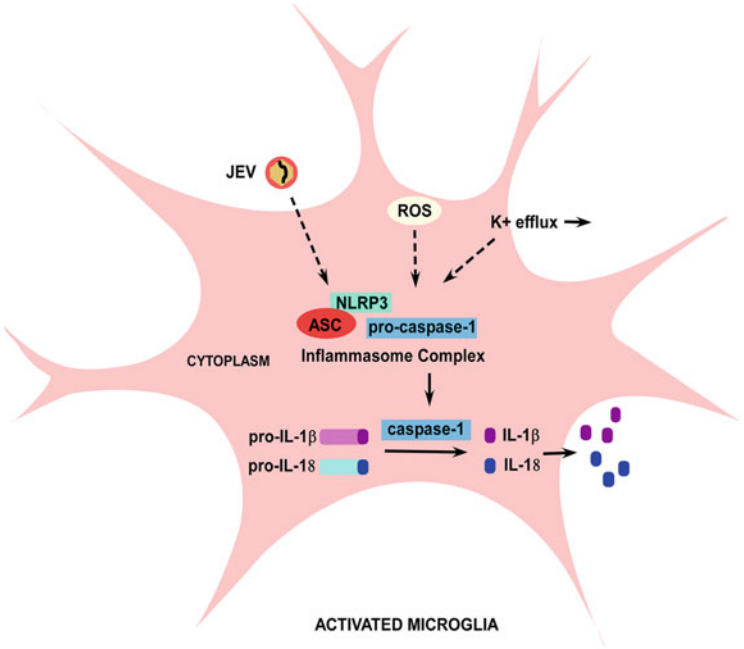

Fig. 6 JEV infection in microglia leads to production of IL-1β and IL-18. NLRP3 is involved in the identification of JEV in microglia. NLRP3 along with adaptor molecule ASC recruits pro-caspase-1 forming the inflammasome, cleaving it into caspase-1 which cleaves pro-IL-1β and pro-IL-18 into IL-1β and IL-18. ROS and K^+ efflux aid in the process of inflammasome formation during JEV infection. (Adapted from Kaushik et al. 2012 PLoS One)

production of IL-1β and IL-18 in case of JEV infection. JEV infection also induces production of ROS which acts as a stress signal for NLRP3 inflammasome complex formation and the subsequent production of these cytokines. Upon using the ROS inhibitor—DPI—in JEV-treated microglial cells, not only the ROS levels were reduced, but also the downstream caspase-1 activity and production of IL-1β and IL-18 were reduced. Apart from ROS, efflux of K^+ also acts as a danger signal in activating the inflammasome complex. Thus, on inhibiting K^+ efflux in JEV-treated microglial cells by growing them in an excess of potassium chloride in the culture media, a significant reduction in caspase-1 activity as well as in levels of IL-1β and IL-18 was seen.

Immunohistochemical studies on 4 days post-JEV-infected mice brain sections depict the release of pro-inflammatory cytokines IL-18 and IL-1β by activated microglia and astrocytes, which are a hallmark of neuroinflammation associated with JEV infection. IL-18 and IL-1β also induce apoptotic molecules in neurons, causing bystander neuronal death (Das et al. 2008). Figure 6 sketches the activation of NLRP3 inflammasome in response to JEV infection.

9.4 HSP60 Bridges JEV-Induced IL-1β Production in Microglia

As it has already been learnt that HSP60 is responsible for IL-1β production in microglia leading to inflammation, we were interested in understanding whether HSP60 had any role to play in IL-1β expression and associated inflammation in the context of JEV infection. JEV stands as an outstanding model to study neuroinflammation; hence we were keen on examining the expression of HSP60 in N9 microglia cells, mice brains and FFPE human brain sections infected with JEV. HSP60 transcripts increased significantly in the above three conditions. An increased expression of HSP60 protein was seen in both N9 microglia and mice brain infected with JEV. Thus, an overall increase in HSP60 was seen in case of JEV infection both at transcript and protein levels. Simultaneously, an increase in IL-1β secretion was also seen in both JEV-infected N9 microglia cells and JEV-infected mice brains compared to their controls by ELISA. This further emphasizes the fact that JEV infection indeed results in an increased HSP60 expression as well as IL-1β secretion.

9.5 Abrogation of HSP60 Amends Inflammatory Cascade

To dissect the role of HSP60 in JEV-induced IL-1β expression in microglia, knockdown studies for HSP60 were done in vitro (N9 cells) and in vivo (mice brain) models. Both in vitro and in vivo models showed a decrease in IL-1β expression detected by ELISA upon knocking down HSP60. To further assess the role of HSP60 in mediating microglial activation inducing inflammation, expression of pro-inflammatory enzymes—iNOS and COX-2—and pro-inflammatory cytokines MCP-1, TNF-α and IL-6 in JEV-infected N9 cells and mice brains with HSP60 knocked down were checked. It was observed that downregulation of HSP60 caused an overall decrease in the expression of all the pro-inflammatory markers mentioned above in both in vitro and in vivo models.

The effect of HSP60 knockdown on the survival of mice was also checked. It was expected that, if knocking down HSP60 in mice could decrease the production of IL-1β as well as other cytokines and lessen the overall inflammation, it might play a role in the survival of infected mice also. To our surprise, it was observed that mice with HSP60 knockdown had increased survival, and it lived longer for about 10 more days compared to the JEV-infected mice with intact HSP60, which died early. This increase in survival of HSP60 knockdown mice can be attributed to the reduction in inflammation caused by knocking down HSP60.

Mice model of JEV shows symptoms like tremors, ruffled fur, hunching, ataxia, hind limb paralysis, motor deficits, etc. Appearance of these symptoms was delayed in HSP60 knockdown JEV-infected mice compared to JEV-infected mice. Behaviour scores were calculated for both the mice groups, which suggested the former group having a better score than the latter group indicating the late onset of symptoms in HSP60 knockdown JEV-infected mice.

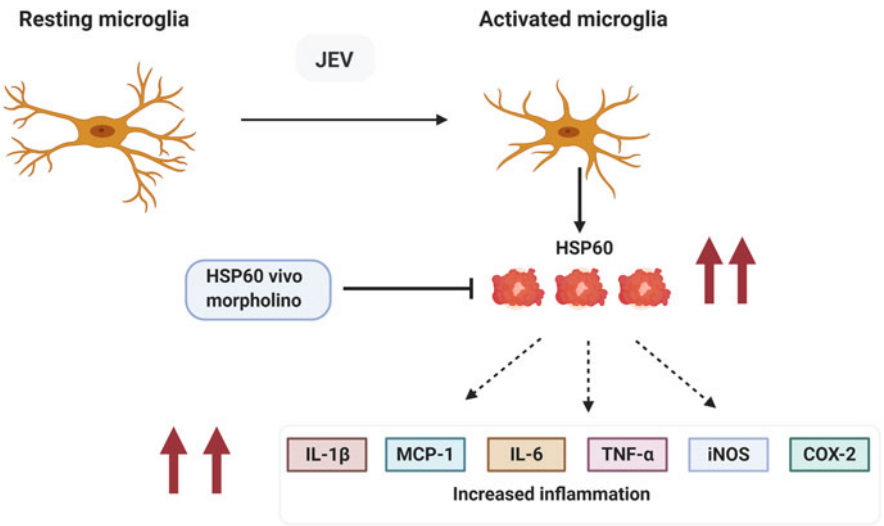

Fig. 7 Schematic diagram showing the role of HSP60 in JEV infection. JEV infection leads to an increased expression of HSP60 which escalates expression of IL-1β along with other pro-inflammatory mediators in activated microglia. Knocking down HSP60 reverses the effect and abrogates profound inflammation. (Figure re-prepared from Shalini Swaroop's PhD thesis) (Created with BioRender.com)

Clearly, JEV infection causes microglial activation and an increased expression of HSP60 in microglia. HSP60 exacerbates neuroinflammation further by stimulating production of pro-inflammatory mediators by activated microglia. Upon downregulating HSP60, IL-1β expression decreases, and inflammation associated with JEV infection subsides causing an increased survival in JEV-infected mice. Another study highlights HSP60 as a potential drug target in case of human hepatitis B virus, where downregulating HSP60 in infected cell blocks HBV replication (Park et al. 2003). Thus, HSP60 has the potential to be a therapeutic target for ameliorating various neuroinflammatory and neurodegenerative diseases, as downregulating it significantly reduces inflammation and improves survival in case of JEV. Figure 7 summarizes the role of HSP60 in JEV infection.

10 Summary

Heat shock proteins (HSPs) are a group of evolutionarily conserved proteins that respond to cellular stress. These proteins act as molecular chaperones and facilitate protein folding inside the cell. HSPs can be broadly classified into as many as seven families on the basis of their molecular weights. HSP60 in particular plays a significant role in many infectious as well as non-infectious diseases, by acting as an immunomodulatory molecule. Previous reports suggested the role of HSP60 as neuron-glia crosstalk molecule during neurodegeneration, serving as a potential

therapeutic target in many neurodegenerative diseases. Also, proteomics data identified HSP60 as a highly interacted protein in N9 microglial cell line stimulated with IL-1β treatment, motivating us to better understand the role of HSP60 in regulating IL-1β-induced microglial activation causing inflammation.

Microglia are the immune sentinels of central nervous system that are always on the lookout for approaching danger signals. Upon facing any assault in the form of stimuli such as pathogens, toxins, stress, etc., microglia get activated. Microglial activation leads to secretion of various pro-inflammatory and anti-inflammatory factors which cause neuroinflammation. The microglial inflammatory response is essential to fight off pathogenic invasion, but an exaggeration of inflammation can affect the health of neurons adversely, causing neurodegeneration. IL-1β is the master regulator of inflammation and plays a fundamental role in activation of microglia as well as in maintaining the neuroinflammation. IL-1β plays a crucial role in many neurodegenerative diseases as well as neuroinflammatory conditions like stroke, brain injury, Alzheimer's disease, Parkinson's disease, cerebral malaria, etc. by orchestrating numerous inflammatory signalling pathways in microglia and thus aggravating inflammation. Moreover, our results also showed microglial as well as mice brain inflammation caused by IL-1β treatment.

We studied the effect of HSP60 on microglial activation inducing inflammation by overexpressing HSP60 in microglial cells. Results indicated an increase in expression of iNOS and COX-2 proteins by western blotting and TNF-α, MCP-1 and IL-6 by cytokine bead array. Knockdown of HSP60 prevented inflammation induced by microglia even in the presence of IL-1β. Therefore, we gained an insight into the mechanism of IL-1β treatment in microglia. IL-1β binds to its cognate receptor IL-1R in microglial cell surface and induces expression of HSP60 in cell cytoplasm. Cytoplasmic HSP60 in turn is secreted out in the extracellular milieu and interacts to TLR4 receptor on microglia. The interaction of HSP60 with TLR4 was determined by co-immunoprecipitation; upon using an inhibitor against TLR4 we saw that there was a compromise in IL-1β-induced inflammation. This emphasized the importance of TLR4 in IL-1β-induced inflammation. Binding of HSP60 with TLR4 activates NLRP3 inflammasome activation and endogenous IL-1β production in microglia. HSP60-TLR4 binding caused an increase in phosphorylation and nuclear translocation of NF-κB, which in turn regulates transcription of pro-IL-1β and NLRP3 genes. HSP60 upon interacting with TLR4 also plays a regulatory role in inducing mitochondrial stress and ROS generation which leads to activation of caspase-1. Caspase-1 enhances the sustained production of IL-1β. IL-1β treatment also induced mitochondrial damage causing generation of ROS in microglia which drives the activation of NLRP3 inflammasome complex formation.

We had already seen the importance of IL-1β as a crucial inflammatory factor in a number of neuroinflammatory and neurodegenerative diseases. Our own findings further strengthened this hypothesis, when we found an increased expression of both IL-1β and HSP60 in various non-infectious and infectious brain inflammatory diseases. Upon exploring the underlying mechanism of IL-1β production, it was seen that HSP60-TLR4 interaction exacerbates neuroinflammation by stimulating

production of endogenous IL-1β in activated microglia by causing induction of NLRP3 pathway. Mitochondrial damage also can trigger NLRP3 inflammasome pathway activation, leading to endogenous IL-1β production in microglia. Hereby, a paramount role of HSP60 as an immunomodulatory molecule has been established by this study. HSP60 induces mitochondrial stress by reducing mitochondrial membrane potential and promoting ROS generation. Additionally, HSP60 also increases the phosphorylation and nuclear localization of NF-κB leading to upregulation of several inflammatory genes, thus connecting mitochondrial stress and inflammation.

Taking another route, TLR4 gets upregulated upon HSP60 binding and in turn activates MyD88 expression. MyD88 phosphorylates MEK3/6, which is an upstream modulator of p38 MAPK. p38 MAPK gets phosphorylated by MEK3/6 and transcribes other pro-inflammatory cytokines—TNF-α, MCP-1 and IL-6—and pro-inflammatory enzymes iNOS and COX-2. The signalling pathway underlying inflammation mediated by microglia upon IL-1β treatment is clear. Based on these findings, we put forth a feed-forward loop of signalling pathway involving HSP60 in IL-1β-induced inflammation in microglial cells. Thus, HSP60 activates microglia in a TLR4-MEK3/6-p38 MAPK-dependent manner. However, there are still many questions left unanswered. For instance, the mechanism that causes HSP60 to phosphorylate NF-κB is unknown. The way by which p38 MAPK induces transcription of various inflammatory genes is yet to be investigated.

In the final part of the study, the role of HSP60 in Japanese encephalitis virus-induced IL-1β inflammation was explored. JEV is a neurotropic virus that invades the CNS after infecting peripheral tissues. Microglia get activated and secrete pro-inflammatory cytokines in response to the virus. JEV induces expression of HSP60 in microglia as well as mice brain. Knocking down HSP60 abrogates inflammation, increasing the survival of JEV-infected BALB/C mice. HSP60 downregulation causes levels of IL-1β and inflammation to reduce in microglia further supporting the fact that HSP60 regulates microglial activation and subsequent IL-1β production in case of JEV infection. HSP60 also comes forward as a potential therapeutic target against inflammation as targeting it can also increase survival and delay the onset of symptoms in case of JEV.

In a nutshell, we could decipher the molecular mechanism behind microglia-mediated inflammation driven by HSP60. In activated microglia IL-1β manages its own production in a HSP60-dependent manner. IL-1β upon binding to its cognate receptor upregulates expression of HSP60 in microglia, which is then secreted outside. Secreted HSP60 binds to TLR4 expressed on microglial surface to activate p38 MAPK and facilitate NF-κB phosphorylation, mitochondrial damage and ROS generation and in turn activate NLRP3 inflammasome to ensure endogenous IL-1β production. JEV increases HSP60 expression and in turn controls inflammasome activation leading to constitutive production of IL-1β, mounting an exaggerated immune response against JEV. Figure 8 gives a diagrammatic overview of the pathways that get activated on HSP60-TLR4 binding in the context of microglia.

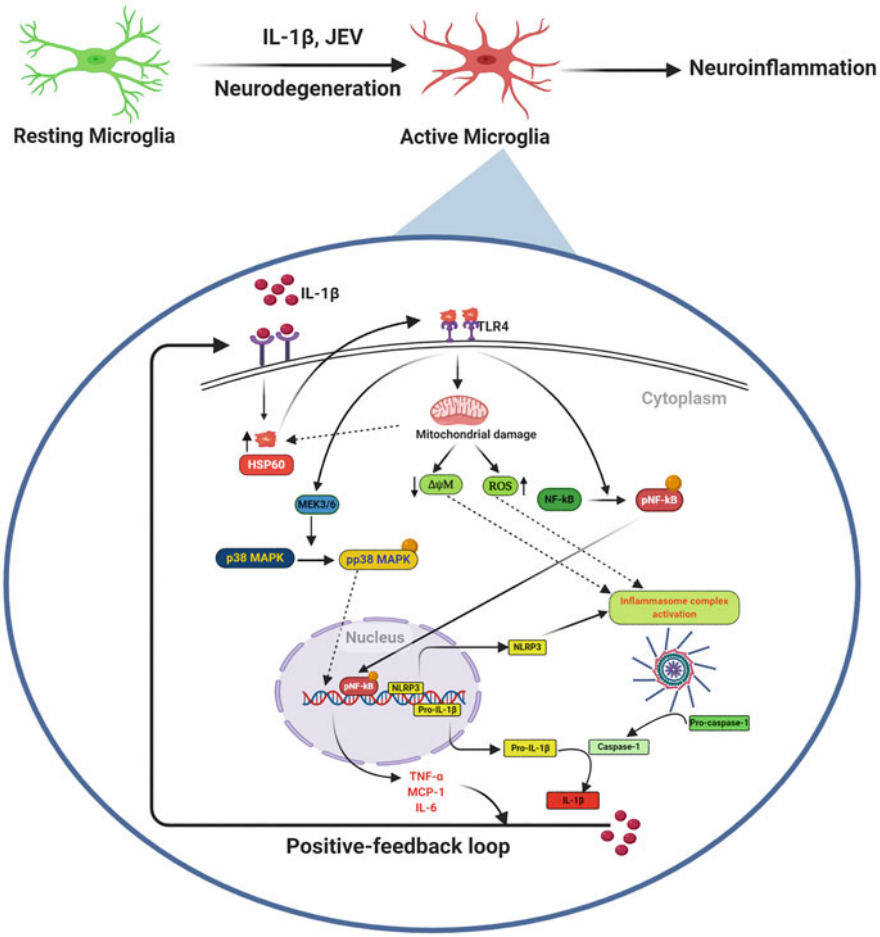

Fig. 8 Interdependent molecular events triggered upon binding of HSP60 to TLR4 on microglial cell surface. IL-1β induces its own production in a HSP60-dependent manner inside activated microglia. IL-1β causes an upregulation of HSP60, which is secreted outside. HSP60 binds to TLR4 causing p38 MAPK pathway activation. HSP60-TLR4 binding causes phosphorylation and nuclear localization of NF-κB, mitochondrial damage and ROS generation which activates inflammasome complex causing IL-1β production endogenously. JEV also aids in the increased production of HSP60, activating the inflammasome complex leading to IL-1β production in a constitutive manner, exaggerating the immune response further. (Created with BioRender.com)

Acknowledgement Authors sincerely thank Prof P. N. Tandon for critically reviewing the chapter and giving insightful comments. The work in AB's laboratory has been generously supported by research grants from the Department of Biotechnology and the Department of Science and Technology. AB is also a recipient of J C Bose Fellowship from the Science and Engineering Research Board, Ministry of Science and Technology.

References

Abulafia-Lapid R, Elias D, Raz I, Keren-Zur Y, Atlan H, Cohen IR (1999) T cell proliferative responses of type 1 diabetes patients and healthy individuals to human hsp60 and its peptides. J Autoimmun 12(2):121–129. https://doi.org/10.1006/jaut.1998.0262

Anders H-J, Muruve DA (2011) The inflammasomes in kidney disease. J Am Soc Nephrol 22 (6):1007–1018. https://doi.org/10.1681/ASN.2010080798

Anraku I, Rajasuriar R, Dobbin C, Brown R, Lewin SR, Suhrbier A (2012) Circulating heat shock protein 60 levels are elevated in HIV patients and are reduced by anti-retroviral therapy. PLoS One 7(9):e45291. https://doi.org/10.1371/journal.pone.0045291

Appel-Cresswell S, Rajput AH, Sossi V, Thompson C, Silva V, McKenzie J, Dinelle K, McCormick SE, Vilariño-Güell C, Stoessl AJ, Dickson DW, Robinson CA, Farrer MJ, Rajput A (2014) Clinical, positron emission tomography, and pathological studies of DNAJC13 p. N855S Parkinsonism. Mov Disord 29(13):1684–1687. https://doi.org/10.1002/mds.26019

Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK (2000) HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6(4):435–442. https://doi.org/10.1038/74697

Bajramović JJ, Bsibsi M, Geutskens SB, Hassankhan R, Verhulst KC, Stege GJ, de Groot CJ, van Noort JM (2000) Differential expression of stress proteins in human adult astrocytes in response to cytokines. J Neuroimmunol 106(1–2):14–22. https://doi.org/10.1016/s0165-5728(99)00260-x

Barboza L, Salmen S, Goncalves L, Colmenares M, Peterson D, Montes H, Cartagirone R, Gutiérrez Mdel C, Berrueta L (2007) Antigen-induced regulatory T cells in HBV chronically infected patients. Virology 368(1):41–49. https://doi.org/10.1016/j.virol.2007.06.030

Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T, Wu J, Monks BG, Fitzgerald KA, Hornung V, Latz E (2009) Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J Immunol 183(2):787–791. https://doi.org/10.4049/jimmunol.0901363

Bayramgürler D, Ozkara SK, Apaydin R, Erçin C, Bilen N (2004) Heat shock proteins 60 and 70 expression of cutaneous lichen planus: comparison with normal skin and psoriasis vulgaris. J Cutan Pathol 31(9):586–594. https://doi.org/10.1111/j.0303-6987.2004.00234.x

Belles C, Kuhl A, Nosheny R, Carding SR (1999) Plasma membrane expression of heat shock protein 60 in vivo in response to infection. Infect Immun 67(8):4191–4200. https://doi.org/10.1128/iai.67.8.4191-4200.1999

Boje KM, Arora PK (1992) Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. Brain Res 587(2):250–256. https://doi.org/10.1016/0006-8993(92)91004-x

Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. Plant Mol Biol 32(1–2):191–222. https://doi.org/10.1007/BF00039383

Bottazzi B, Doni A, Garlanda C, Mantovani A (2010) An integrated view of humoral innate immunity: pentraxins as a paradigm. Annu Rev Immunol 28:157–183. https://doi.org/10.1146/annurev-immunol-030409-101305

Braig K, Otwinowski Z, Hegde R, Boisvert DC, Joachimiak A, Horwich AL, Sigler PB (1994) The crystal structure of the bacterial chaperonin GroEL at 2.8 A. Nature 371(6498):578–586. https://doi.org/10.1038/371578a0

Brudzynski K, Martinez V, Gupta RS (1992) Immunocytochemical localization of heat-shock protein 60-related protein in beta-cell secretory granules and its altered distribution in non-obese diabetic mice. Diabetologia 35(4):316–324. https://doi.org/10.1007/bf00401198

Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo AP, Kroemer G, Solary E, Garrido C (2000) Hsp27 negatively regulates cell death by interacting with cytochrome c. Nat Cell Biol 2(9):645–652. https://doi.org/10.1038/35023595

Buzzard KA, Giaccia AJ, Killender M, Anderson RL (1998) Heat shock protein 72 modulates pathways of stress-induced apoptosis. J Biol Chem 273(27):17147–17153. https://doi.org/10.1074/jbc.273.27.17147

Caplan AJ, Cyr DM, Douglas MG (1993) Eukaryotic homologues of Escherichia coli dnaJ: a diverse protein family that functions with hsp70 stress proteins. Mol Biol Cell 4(6):555–563. https://doi.org/10.1091/mbc.4.6.555

Capponi S, Geroldi A, Fossa P, Grandis M, Ciotti P, Gulli R, Schenone A, Mandich P, Bellone E (2011) HSPB1 and HSPB8 in inherited neuropathies: study of an Italian cohort of dHMN and CMT2 patients. J Peripher Nerv Syst 16(4):287–294. https://doi.org/10.1111/j.1529-8027.2011.00361.x

Cargnello M, Roux PP (2011) Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev 75(1):50–83. https://doi.org/10.1128/mmbr.00031-10

Cechetto JD, Soltys BJ, Gupta RS (2000) Localization of mitochondrial 60-kD heat shock chaperonin protein (Hsp60) in pituitary growth hormone secretory granules and pancreatic zymogen granules. J Histochem Cytochem 48(1):45–56. https://doi.org/10.1177/002215540004800105

Chen S, Roseman AM, Hunter AS, Wood SP, Burston SG, Ranson NA, Clarke AR, Saibil HR (1994) Location of a folding protein and shape changes in GroEL-GroES complexes imaged by cryo-electron microscopy. Nature 371(6494):261–264. https://doi.org/10.1038/371261a0

Chen W, Syldath U, Bellmann K, Burkart V, Kolb H (1999) Human 60-kDa heat-shock protein: a danger signal to the innate immune system. J Immunol 162(6):3212–3219

Chen CJ, Ou YC, Lin SY, Raung SL, Liao SL, Lai CY, Chen SY, Chen JH (2010) Glial activation involvement in neuronal death by Japanese encephalitis virus infection. J Gen Virol 91(Pt 4):1028–1037. https://doi.org/10.1099/vir.0.013565-0

Chen YS, Chang CW, Tsay YG, Huang LY, Wu YC, Cheng LH, Yang CC, Wu CH, Teo WH, Hung KF, Huang CY, Lee TC, Lo JF (2018) HSP40 co-chaperone protein Tid1 suppresses metastasis of head and neck cancer by inhibiting Galectin-7-TCF3-MMP9 axis signaling. Theranostics 8(14):3841–3855. https://doi.org/10.7150/thno.25784

Cheng MY, Hartl FU, Martin J, Pollock RA, Kalousek F, Neupert W, Hallberg EM, Hallberg RL, Horwich AL (1989) Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. Nature 337(6208):620–625. https://doi.org/10.1038/337620a0

Cheng YC, Huang CJ, Lee YJ, Tien LT, Ku WC, Chien R, Lee FK, Chien CC (2016) Knocking down of heat-shock protein 27 directs differentiation of functional glutamatergic neurons from placenta-derived multipotent cells. Sci Rep 6:30314. https://doi.org/10.1038/srep30314

Cohen-Sfady M, Nussbaum G, Pevsner-Fischer M, Mor F, Carmi P, Zanin-Zhorov A, Lider O, Cohen IR (2005) Heat shock protein 60 activates B cells via the TLR4-MyD88 pathway. J Immunol 175(6):3594–3602. https://doi.org/10.4049/jimmunol.175.6.3594

Cohen-Sfady M, Pevsner-Fischer M, Margalit R, Cohen IR (2009) Heat shock protein 60, via MyD88 innate signaling, protects B cells from apoptosis, spontaneous and induced. J Immunol 183(2):890–896. https://doi.org/10.4049/jimmunol.0804238

Cowan KJ, Storey KB (2003) Mitogen-activated protein kinases: new signaling pathways functioning in cellular responses to environmental stress. J Exp Biol 206(Pt 7):1107–1115. https://doi.org/10.1242/jeb.00220

Csermely P, Schnaider T, Soti C, Prohászka Z, Nardai G (1998) The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. Pharmacol Ther 79(2):129–168. https://doi.org/10.1016/s0163-7258(98)00013-8

Culbert AA, Skaper SD, Howlett DR, Evans NA, Facci L, Soden PE, Seymour ZM, Guillot F, Gaestel M, Richardson JC (2006) MAPK-activated protein kinase 2 deficiency in microglia inhibits pro-inflammatory mediator release and resultant neurotoxicity. Relevance to neuroinflammation in a transgenic mouse model of Alzheimer disease. J Biol Chem 281(33):23658–23667. https://doi.org/10.1074/jbc.M513646200

D'Souza SM, Brown IR (1998) Constitutive expression of heat shock proteins Hsp90, Hsc70, Hsp70 and Hsp60 in neural and non-neural tissues of the rat during postnatal development. Cell Stress Chaperones 3(3):188–199. https://doi.org/10.1379/1466-1268(1998)003<0188:ceohsp>2.3.co;2

Das S, Basu A (2008) Japanese encephalitis virus infects neural progenitor cells and decreases their proliferation. J Neurochem 106(4):1624–1636. https://doi.org/10.1111/j.1471-4159.2008.05511.x

Das S, Mishra MK, Ghosh J, Basu A (2008) Japanese Encephalitis Virus infection induces IL-18 and IL-1beta in microglia and astrocytes: correlation with in vitro cytokine responsiveness of glial cells and subsequent neuronal death. J Neuroimmunol 195(1–2):60–72. https://doi.org/10.1016/j.jneuroim.2008.01.009

Daugaard M, Rohde M, Jäättelä M (2007) The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. FEBS Lett 581(19):3702–3710. https://doi.org/10.1016/j.febslet.2007.05.039

Ding F, Li F, Li Y, Hou X, Ma Y, Zhang N, Ma J, Zhang R, Lang B, Wang H, Wang Y (2016a) HSP60 mediates the neuroprotective effects of curcumin by suppressing microglial activation. Exp Ther Med 12(2):823–828. https://doi.org/10.3892/etm.2016.3413

Ding F, Li Y, Hou X, Zhang R, Hu S, Wang Y (2016b) Oxymatrine inhibits microglia activation via HSP60-TLR4 signaling. Biomed Rep 5(5):623–628. https://doi.org/10.3892/br.2016.776

Doyle SM, Wickner S (2009) Hsp104 and ClpB: protein disaggregating machines. Trends Biochem Sci 34(1):40–48. https://doi.org/10.1016/j.tibs.2008.09.010

Falsone SF, Kungl AJ, Rek A, Cappai R, Zangger K (2009) The molecular chaperone Hsp90 modulates intermediate steps of amyloid assembly of the Parkinson-related protein alpha-synuclein. J Biol Chem 284(45):31190–31199. https://doi.org/10.1074/jbc.M109.057240

Felip E, Barlesi F, Besse B, Chu Q, Gandhi L, Kim SW, Carcereny E, Sequist LV, Brunsvig P, Chouaid C, Smit EF, Groen HJM, Kim DW, Park K, Avsar E, Szpakowski S, Akimov M, Garon EB (2018) Phase 2 study of the HSP-90 inhibitor AUY922 in previously treated and molecularly defined patients with advanced non-small cell lung cancer. J Thorac Oncol 13(4):576–584. https://doi.org/10.1016/j.jtho.2017.11.131

Feng H, Zeng Y, Graner MW, Katsanis E (2002) Stressed apoptotic tumor cells stimulate dendritic cells and induce specific cytotoxic T cells. Blood 100(12):4108–4115. https://doi.org/10.1182/blood-2002-05-1389

Feng M, Zhang L, Liu Z, Zhou P, Lu X (2013) The expression and release of Hsp60 in 6-OHDA induced in vivo and in vitro models of Parkinson's disease. Neurochem Res 38(10):2180–2189. https://doi.org/10.1007/s11064-013-1127-8

Fenton WA, Kashi Y, Furtak K, Horwich AL (1994) Residues in chaperonin GroEL required for polypeptide binding and release. Nature 371(6498):614–619. https://doi.org/10.1038/371614a0

Flohé SB, Brüggemann J, Lendemans S, Nikulina M, Meierhoff G, Flohé S, Kolb H (2003) Human heat shock protein 60 induces maturation of dendritic cells versus a Th1-promoting phenotype. J Immunol 170(5):2340–2348. https://doi.org/10.4049/jimmunol.170.5.2340

Garrido C, Bruey JM, Fromentin A, Hammann A, Arrigo AP, Solary E (1999) HSP27 inhibits cytochrome c-dependent activation of procaspase-9. FASEB J 13(14):2061–2070. https://doi.org/10.1096/fasebj.13.14.2061

Ghosh D, Basu A (2009) Japanese encephalitis-a pathological and clinical perspective. PLoS Negl Trop Dis 3(9):e437. https://doi.org/10.1371/journal.pntd.0000437

Ghoshal A, Das S, Ghosh S, Mishra MK, Sharma V, Koli P, Sen E, Basu A (2007) Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis. Glia 55(5):483–496. https://doi.org/10.1002/glia.20474

Glaessgen A, Jonmarker S, Lindberg A, Nilsson B, Lewensohn R, Ekman P, Valdman A, Egevad L (2008) Heat shock proteins 27, 60 and 70 as prognostic markers of prostate cancer. APMIS 116(10):888–895. https://doi.org/10.1111/j.1600-0463.2008.01051.x

Gobert AP, Bambou JC, Werts C, Balloy V, Chignard M, Moran AP, Ferrero RL (2004) Helicobacter pylori heat shock protein 60 mediates interleukin-6 production by macrophages

via a toll-like receptor (TLR)-2-, TLR-4-, and myeloid differentiation factor 88-independent mechanism. J Biol Chem 279(1):245–250. https://doi.org/10.1074/jbc.M307858200

Graef KM, Vreede FT, Lau YF, McCall AW, Carr SM, Subbarao K, Fodor E (2010) The PB2 subunit of the influenza virus RNA polymerase affects virulence by interacting with the mitochondrial antiviral signaling protein and inhibiting expression of beta interferon. J Virol 84(17):8433–8445. https://doi.org/10.1128/jvi.00879-10

Guan Z, Buckman SY, Pentland AP, Templeton DJ, Morrison AR (1998) Induction of cyclooxygenase-2 by the activated MEKK1 --> SEK1/MKK4 --> p38 mitogen-activated protein kinase pathway. J Biol Chem 273(21):12901–12908. https://doi.org/10.1074/jbc.273.21.12901

Gupta S, Knowlton AA (2002) Cytosolic heat shock protein 60, hypoxia, and apoptosis. Circulation 106(21):2727–2733. https://doi.org/10.1161/01.cir.0000038112.64503.6e

Hanke ML, Kielian T (2011) Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential. Clin Sci (Lond) 121(9):367–387. https://doi.org/10.1042/cs20110164

Hazra B, Chakraborty S, Bhaskar M, Mukherjee S, Mahadevan A, Basu A (2019) miR-301a regulates inflammatory response to Japanese encephalitis virus infection via suppression of NKRF activity. J Immunol 203(8):2222–2238. https://doi.org/10.4049/jimmunol.1900003

Hoshino T, Murao N, Namba T, Takehara M, Adachi H, Katsuno M, Sobue G, Matsushima T, Suzuki T, Mizushima T (2011) Suppression of Alzheimer's disease-related phenotypes by expression of heat shock protein 70 in mice. J Neurosci 31(14):5225–5234. https://doi.org/10.1523/jneurosci.5478-10.2011

Houenou LJ, Li L, Lei M, Kent CR, Tytell M (1996) Exogenous heat shock cognate protein Hsc 70 prevents axotomy-induced death of spinal sensory neurons. Cell Stress Chaperones 1(3):161–166. https://doi.org/10.1379/1466-1268(1996)001<0161:ehscph>2.3.co;2

Hu WC (2016) Microarray analysis of PBMC after Plasmodium falciparum infection: molecular insights into disease pathogenesis. Asian Pac J Trop Med 9(4):313–323. https://doi.org/10.1016/j.apjtm.2016.03.013

Hu X, Leak RK, Shi Y, Suenaga J, Gao Y, Zheng P, Chen J (2015) Microglial and macrophage polarization—new prospects for brain repair. Nat Rev Neurol 11(1):56–64. https://doi.org/10.1038/nrneurol.2014.207

Imamura Y, Kurokawa MS, Yoshikawa H, Nara K, Takada E, Masuda C, Tsukikawa S, Ozaki S, Matsuda T, Suzuki N (2005) Involvement of Th1 cells and heat shock protein 60 in the pathogenesis of intestinal Behcet's disease. Clin Exp Immunol 139(2):371–378. https://doi.org/10.1111/j.1365-2249.2005.02695.x

Inose Y, Kato Y, Kitagawa K, Uchiyama S, Shibata N (2015) Activated microglia in ischemic stroke penumbra upregulate MCP-1 and CCR2 expression in response to lysophosphatidylcholine derived from adjacent neurons and astrocytes. Neuropathology 35(3):209–223. https://doi.org/10.1111/neup.12182

Jakob U, Gaestel M, Engel K, Buchner J (1993) Small heat shock proteins are molecular chaperones. J Biol Chem 268(3):1517–1520

Jander S, Pohl J, D'Urso D, Gillen C, Stoll G (1998) Time course and cellular localization of interleukin-10 mRNA and protein expression in autoimmune inflammation of the rat central nervous system. Am J Pathol 152(4):975–982

Kakimura J, Kitamura Y, Takata K, Umeki M, Suzuki S, Shibagaki K, Taniguchi T, Nomura Y, Gebicke-Haerter PJ, Smith MA, Perry G, Shimohama S (2002) Microglial activation and amyloid-beta clearance induced by exogenous heat-shock proteins. FASEB J 16(6):601–603. https://doi.org/10.1096/fj.01-0530fje

Kamphuis S, Kuis W, de Jager W, Teklenburg G, Massa M, Gordon G, Boerhof M, Rijkers GT, Uiterwaal CS, Otten HG, Sette A, Albani S, Prakken BJ (2005) Tolerogenic immune responses to novel T-cell epitopes from heat-shock protein 60 in juvenile idiopathic arthritis. Lancet 366(9479):50–56. https://doi.org/10.1016/s0140-6736(05)66827-4

Kanneganti TD, Body-Malapel M, Amer A, Park JH, Whitfield J, Franchi L, Taraporewala ZF, Miller D, Patton JT, Inohara N, Núñez G (2006) Critical role for Cryopyrin/Nalp3 in activation

of caspase-1 in response to viral infection and double-stranded RNA. J Biol Chem 281(48): 36560–36568. https://doi.org/10.1074/jbc.M607594200

Kant Upadhyay R (2013) Biomarkers in Japanese encephalitis: a review. Biomed Res Int 2013: 591290. https://doi.org/10.1155/2013/591290

Kaushik DK, Gupta M, Das S, Basu A (2010) Krüppel-like factor 4, a novel transcription factor regulates microglial activation and subsequent neuroinflammation. J Neuroinflammation 7:68. https://doi.org/10.1186/1742-2094-7-68

Kaushik DK, Gupta M, Kumawat KL, Basu A (2012) NLRP3 inflammasome: key mediator of neuroinflammation in murine Japanese encephalitis. PLoS One 7(2):e32270. https://doi.org/10.1371/journal.pone.0032270

Kaushik DK, Thounaojam MC, Kumawat KL, Gupta M, Basu A (2013) Interleukin-1β orchestrates underlying inflammatory responses in microglia via Krüppel-like factor 4. J Neurochem 127(2): 233–244. https://doi.org/10.1111/jnc.12382

Kawasaki T, Kawai T (2014) Toll-like receptor signaling pathways. Front Immunol 5:461. https://doi.org/10.3389/fimmu.2014.00461

Keskin O, Bahar I, Flatow D, Covell DG, Jernigan RL (2002) Molecular mechanisms of chaperonin GroEL-GroES function. Biochemistry 41(2):491–501. https://doi.org/10.1021/bi011393x

Khadir A, Kavalakatt S, Cherian P, Warsame S, Abubaker JA, Dehbi M, Tiss A (2018) Physical exercise enhanced heat shock protein 60 expression and attenuated inflammation in the adipose tissue of human diabetic obese. Front Endocrinol (Lausanne) 9:16. https://doi.org/10.3389/fendo.2018.00016

Khan MN, Shukla D, Bansal A, Mustoori S, Ilavazhagan G (2009) Immunogenicity and protective efficacy of GroEL (hsp60) of Streptococcus pneumoniae against lethal infection in mice. FEMS Immunol Med Microbiol 56(1):56–62. https://doi.org/10.1111/j.1574-695X.2009.00548.x

Kiefer R, Schweitzer T, Jung S, Toyka KV, Hartung HP (1998) Sequential expression of transforming growth factor-beta1 by T-cells, macrophages, and microglia in rat spinal cord during autoimmune inflammation. J Neuropathol Exp Neurol 57(5):385–395. https://doi.org/10.1097/00005072-199805000-00002

Koll H, Guiard B, Rassow J, Ostermann J, Horwich AL, Neupert W, Hartl FU (1992) Antifolding activity of hsp60 couples protein import into the mitochondrial matrix with export to the intermembrane space. Cell 68(6):1163–1175. https://doi.org/10.1016/0092-8674(92)90086-r

Krementsov DN, Thornton TM, Teuscher C, Rincon M (2013) The emerging role of p38 mitogen-activated protein kinase in multiple sclerosis and its models. Mol Cell Biol 33(19):3728–3734. https://doi.org/10.1128/mcb.00688-13

Kumar R, Mathur A, Kumar A, Sharma S, Chakraborty S, Chaturvedi UC (1990) Clinical features & prognostic indicators of Japanese encephalitis in children in Lucknow (India). Indian J Med Res 91:321–327

Kyriakis JM, Avruch J (2012) Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. Physiol Rev 92(2):689–737. https://doi.org/10.1152/physrev.00028.2011

Lehnardt S, Lachance C, Patrizi S, Lefebvre S, Follett PL, Jensen FE, Rosenberg PA, Volpe JJ, Vartanian T (2002) The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. J Neurosci 22(7):2478–2486. https://doi.org/10.1523/jneurosci.22-07-02478.2002

Lehnardt S, Schott E, Trimbuch T, Laubisch D, Krueger C, Wulczyn G, Nitsch R, Weber JR (2008) A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. J Neurosci 28(10):2320–2331. https://doi.org/10.1523/jneurosci.4760-07.2008

Li Y, Zhang R, Hou X, Zhang Y, Ding F, Li F, Yao Y, Wang Y (2017) Microglia activation triggers oligodendrocyte precursor cells apoptosis via HSP60. Mol Med Rep 16(1):603–608. https://doi.org/10.3892/mmr.2017.6673

Lin KM, Lin B, Lian IY, Mestril R, Scheffler IE, Dillmann WH (2001) Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial

function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. Circulation 103(13):1787–1792. https://doi.org/10.1161/01.cir.103.13.1787

Lin FS, Lin CC, Chien CS, Luo SF, Yang CM (2005) Involvement of p42/p44 MAPK, JNK, and NF-kappaB in IL-1beta-induced ICAM-1 expression in human pulmonary epithelial cells. J Cell Physiol 202(2):464–473. https://doi.org/10.1002/jcp.20142

Lin Z, Madan D, Rye HS (2008) GroEL stimulates protein folding through forced unfolding. Nat Struct Mol Biol 15(3):303–311. https://doi.org/10.1038/nsmb.1394

Liu JS, Amaral TD, Brosnan CF, Lee SC (1998) IFNs are critical regulators of IL-1 receptor antagonist and IL-1 expression in human microglia. J Immunol 161(4):1989–1996

Liu T, Zhang L, Joo D, Sun SC (2017) NF-κB signaling in inflammation. Signal Transduct Target Ther 2:17023. https://doi.org/10.1038/sigtrans.2017.23

Lo Bianco C, Shorter J, Régulier E, Lashuel H, Iwatsubo T, Lindquist S, Aebischer P (2008) Hsp104 antagonizes alpha-synuclein aggregation and reduces dopaminergic degeneration in a rat model of Parkinson disease. J Clin Invest 118(9):3087–3097. https://doi.org/10.1172/jci35781

Locke M, Noble EG, Atkinson BG (1990) Exercising mammals synthesize stress proteins. Am J Phys 258(4 Pt 1):C723–C729. https://doi.org/10.1152/ajpcell.1990.258.4.C723

Loones MT, Chang Y, Morange M (2000) The distribution of heat shock proteins in the nervous system of the unstressed mouse embryo suggests a role in neuronal and non-neuronal differentiation. Cell Stress Chaperones 5(4):291–305. https://doi.org/10.1379/1466-1268(2000)005<0291:tdohsp>2.0.co;2

Lorenzo-Betancor O, Ogaki K, Soto-Ortolaza AI, Labbe C, Walton RL, Strongosky AJ, van Gerpen JA, Uitti RJ, McLean PJ, Springer W, Siuda J, Opala G, Krygowska-Wajs A, Barcikowska M, Czyzewski K, McCarthy A, Lynch T, Puschmann A, Rektorova I, Sanotsky Y, Vilariño-Güell C, Farrer MJ, Ferman TJ, Boeve BF, Petersen RC, Parisi JE, Graff-Radford NR, Dickson DW, Wszolek ZK, Ross OA (2015) DNAJC13 p.Asn855Ser mutation screening in Parkinson's disease and pathologically confirmed Lewy body disease patients. Eur J Neurol 22(9):1323–1325. https://doi.org/10.1111/ene.12770

Lu N, Malemud CJ (2019) Extracellular signal-regulated kinase: a regulator of cell growth, inflammation, chondrocyte and bone cell receptor-mediated gene expression. Int J Mol Sci 20(15):doi:10.3390/ijms20153792

Magen D, Georgopoulos C, Bross P, Ang D, Segev Y, Goldsher D, Nemirovski A, Shahar E, Ravid S, Luder A, Heno B, Gershoni-Baruch R, Skorecki K, Mandel H (2008) Mitochondrial hsp60 chaperonopathy causes an autosomal-recessive neurodegenerative disorder linked to brain hypomyelination and leukodystrophy. Am J Hum Genet 83(1):30–42. https://doi.org/10.1016/j.ajhg.2008.05.016

Mandich P, Grandis M, Varese A, Geroldi A, Acquaviva M, Ciotti P, Gulli R, Doria-Lamba L, Fabrizi GM, Giribaldi G, Pizzuti A, Schenone A, Bellone E (2010) Severe neuropathy after diphtheria-tetanus-pertussis vaccination in a child carrying a novel frame-shift mutation in the small heat-shock protein 27 gene. J Child Neurol 25(1):107–109. https://doi.org/10.1177/0883073809334387

Marino C, Krishnan B, Cappello F, Taglialatela G (2019) Hsp60 protects against amyloid β oligomer synaptic toxicity via modification of toxic oligomer conformation. ACS Chem Neurosci 10(6):2858–2867. https://doi.org/10.1021/acschemneuro.9b00086

Martin J, Langer T, Boteva R, Schramel A, Horwich AL, Hartl FU (1991) Chaperonin-mediated protein folding at the surface of groEL through a 'molten globule'-like intermediate. Nature 352(6330):36–42. https://doi.org/10.1038/352036a0

Martinon F, Burns K, Tschopp J (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 10(2):417–426. https://doi.org/10.1016/s1097-2765(02)00599-3

Masgras I, Sanchez-Martin C, Colombo G, Rasola A (2017) The chaperone TRAP1 as a modulator of the mitochondrial adaptations in cancer cells. Front Oncol 7:58. https://doi.org/10.3389/fonc.2017.00058

Mayr M, Metzler B, Kiechl S, Willeit J, Schett G, Xu Q, Wick G (1999) Endothelial cytotoxicity mediated by serum antibodies to heat shock proteins of Escherichia coli and Chlamydia pneumoniae: immune reactions to heat shock proteins as a possible link between infection and atherosclerosis. Circulation 99(12):1560–1566. https://doi.org/10.1161/01.cir.99.12.1560

Melle C, Bogumil R, Ernst G, Schimmel B, Bleul A, von Eggeling F (2006) Detection and identification of heat shock protein 10 as a biomarker in colorectal cancer by protein profiling. Proteomics 6(8):2600–2608. https://doi.org/10.1002/pmic.200500427

Minowada G, Welch WJ (1995) Clinical implications of the stress response. J Clin Invest 95(1): 3–12. https://doi.org/10.1172/JCI117655

Miron T, Wilchek M, Geiger B (1988) Characterization of an inhibitor of actin polymerization in vinculin-rich fraction of turkey gizzard smooth muscle. Eur J Biochem 178(2):543–553. https://doi.org/10.1111/j.1432-1033.1988.tb14481.x

Mishra MK, Basu A (2008) Minocycline neuroprotects, reduces microglial activation, inhibits caspase 3 induction, and viral replication following Japanese encephalitis. J Neurochem 105(5):1582–1595. https://doi.org/10.1111/j.1471-4159.2008.05238.x

Mishra MK, Kumawat KL, Basu A (2008) Japanese encephalitis virus differentially modulates the induction of multiple pro-inflammatory mediators in human astrocytoma and astroglioma cell-lines. Cell Biol Int 32(12):1506–1513. https://doi.org/10.1016/j.cellbi.2008.08.020

Monie TP, Bryant CE, Gay NJ (2009) Activating immunity: lessons from the TLRs and NLRs. Trends Biochem Sci 34(11):553–561. https://doi.org/10.1016/j.tibs.2009.06.011

Ni M, Zhou H, Wey S, Baumeister P, Lee AS (2009) Regulation of PERK signaling and leukemic cell survival by a novel cytosolic isoform of the UPR regulator GRP78/BiP. PLoS One 4(8): e6868. https://doi.org/10.1371/journal.pone.0006868

Noelker C, Morel L, Osterloh A, Alvarez-Fischer D, Lescot T, Breloer M, Gold M, Oertel WH, Henze C, Michel PP, Dodel RC, Lu L, Hirsch EC, Hunot S, Hartmann A (2014) Heat shock protein 60: an endogenous inducer of dopaminergic cell death in Parkinson disease. J Neuroinflammation 11:86. https://doi.org/10.1186/1742-2094-11-86

Noll A, AutenriethIb (1996) Immunity against Yersinia enterocolitica by vaccination with Yersinia HSP60 immunostimulating complexes or Yersinia HSP60 plus interleukin-12. Infect Immun 64(8):2955–2961. https://doi.org/10.1128/iai.64.8.2955-2961.1996

Nour AM, Reichelt M, Ku CC, Ho MY, Heineman TC, Arvin AM (2011) Varicella-zoster virus infection triggers formation of an interleukin-1β (IL-1β)-processing inflammasome complex. J Biol Chem 286(20):17921–17933. https://doi.org/10.1074/jbc.M110.210575

Obata T, Brown GE, Yaffe MB (2000) MAP kinase pathways activated by stress: the p38 MAPK pathway. Crit Care Med 28(4 Suppl):N67–N77. https://doi.org/10.1097/00003246-200004001-00008

Ostermann J, Horwich AL, Neupert W, Hartl FU (1989) Protein folding in mitochondria requires complex formation with hsp60 and ATP hydrolysis. Nature 341(6238):125–130. https://doi.org/10.1038/341125a0

Parag HA, Raboy B, Kulka RG (1987) Effect of heat shock on protein degradation in mammalian cells: involvement of the ubiquitin system. EMBO J 6(1):55–61

Park SG, Lim SO, Jung G (2002) Binding site analysis of human HBV pol for molecular chaperonin, hsp60. Virology 298(1):116–123. https://doi.org/10.1006/viro.2002.1496

Park SG, Lee SM, Jung G (2003) Antisense oligodeoxynucleotides targeted against molecular chaperonin Hsp60 block human hepatitis B virus replication. J Biol Chem 278(41): 39851–39857. https://doi.org/10.1074/jbc.M301618200

Parnas A, Nadler M, Nisemblat S, Horovitz A, Mandel H, Azem A (2009) The MitCHAP-60 disease is due to entropic destabilization of the human mitochondrial Hsp60 oligomer. J Biol Chem 284(41):28198–28203. https://doi.org/10.1074/jbc.M109.031997

Pfister G, Stroh CM, Perschinka H, Kind M, Knoflach M, Hinterdorfer P, Wick G (2005) Detection of HSP60 on the membrane surface of stressed human endothelial cells by atomic force and confocal microscopy. J Cell Sci 118(Pt 8):1587–1594. https://doi.org/10.1242/jcs.02292

Picard D (2002) Heat-shock protein 90, a chaperone for folding and regulation. Cell Mol Life Sci 59(10):1640–1648. https://doi.org/10.1007/pl00012491

Qiu XB, Shao YM, Miao S, Wang L (2006) The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. Cell Mol Life Sci 63(22):2560–2570. https://doi.org/10.1007/s00018-006-6192-6

Quraishe S, Asuni A, Boelens WC, O'Connor V, Wyttenbach A (2008) Expression of the small heat shock protein family in the mouse CNS: differential anatomical and biochemical compartmentalization. Neuroscience 153(2):483–491. https://doi.org/10.1016/j.neuroscience.2008.01.058

Rajan JV, Rodriguez D, Miao EA, Aderem A (2011) The NLRP3 inflammasome detects encephalomyocarditis virus and vesicular stomatitis virus infection. J Virol 85(9):4167–4172. https://doi.org/10.1128/jvi.01687-10

Ran Q, Wadhwa R, Kawai R, Kaul SC, Sifers RN, Bick RJ, Smith JR, Pereira-Smith OM (2000) Extramitochondrial localization of mortalin/mthsp70/PBP74/GRP75. Biochem Biophys Res Commun 275(1):174–179. https://doi.org/10.1006/bbrc.2000.3237

Ranson NA, Farr GW, Roseman AM, Gowen B, Fenton WA, Horwich AL, Saibil HR (2001) ATP-bound states of GroEL captured by cryo-electron microscopy. Cell 107(7):869–879. https://doi.org/10.1016/s0092-8674(01)00617-1

Ritossa FM (1964) Experimental activation of specific loci in polytene chromosomes of Drosophila. Exp Cell Res 35:601–607. https://doi.org/10.1016/0014-4827(64)90147-8

Rosenberger K, Dembny P, Derkow K, Engel O, Krüger C, Wolf SA, Kettenmann H, Schott E, Meisel A, Lehnardt S (2015) Intrathecal heat shock protein 60 mediates neurodegeneration and demyelination in the CNS through a TLR4- and MyD88-dependent pathway. Mol Neurodegener 10:5. https://doi.org/10.1186/s13024-015-0003-1

Rothwell NJ, Luheshi GN (2000) Interleukin 1 in the brain: biology, pathology and therapeutic target. Trends Neurosci 23(12):618–625. https://doi.org/10.1016/s0166-2236(00)01661-1

Saha RN, Jana M, Pahan K (2007) MAPK p38 regulates transcriptional activity of NF-kappaB in primary human astrocytes via acetylation of p65. J Immunol 179(10):7101–7109. https://doi.org/10.4049/jimmunol.179.10.7101

Saibil HR, Zheng D, Roseman AM, Hunter AS, Watson GM, Chen S, Auf Der Mauer A, O'Hara BP, Wood SP, Mann NH, Barnett LK, Ellis RJ (1993) ATP induces large quaternary rearrangements in a cage-like chaperonin structure. Curr Biol 3(5):265–273. https://doi.org/10.1016/0960-9822(93)90176-o

Saito Y, Yamagishi N, Hatayama T (2007) Different localization of Hsp105 family proteins in mammalian cells. Exp Cell Res 313(17):3707–3717. https://doi.org/10.1016/j.yexcr.2007.06.009

Saleh A, Srinivasula SM, Balkir L, Robbins PD, Alnemri ES (2000) Negative regulation of the Apaf-1 apoptosome by Hsp70. Nat Cell Biol 2(8):476–483. https://doi.org/10.1038/35019510

Samali A, Cai J, Zhivotovsky B, Jones DP, Orrenius S (1999) Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. EMBO J 18(8):2040–2048. https://doi.org/10.1093/emboj/18.8.2040

Satoh J, Yukitake M, Kuroda Y (1998) Constitutive and heat-inducible expression of HSP105 in neurons and glial cells in culture. Neuroreport 9(13):2977–2983. https://doi.org/10.1097/00001756-199809140-00011

Sen R, Smale ST (2010) Selectivity of the NF-{kappa}B response. Cold Spring Harb Perspect Biol 2(4):a000257. https://doi.org/10.1101/cshperspect.a000257

Seth RB, Sun L, Ea CK, Chen ZJ (2005) Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 122(5):669–682. https://doi.org/10.1016/j.cell.2005.08.012

Shaul YD, Seger R (2007) The MEK/ERK cascade: from signaling specificity to diverse functions. Biochim Biophys Acta 1773(8):1213–1226. https://doi.org/10.1016/j.bbamcr.2006.10.005

Shiber A, Ravid T (2014) Chaperoning proteins for destruction: diverse roles of Hsp70 chaperones and their co-chaperones in targeting misfolded proteins to the proteasome. Biomol Ther 4(3):704–724. https://doi.org/10.3390/biom4030704

Shin H, Jeon J, Lee JH, Jin S, Ha UH (2017) Pseudomonas aeruginosa GroEL stimulates production of PTX3 by activating the NF-κB pathway and simultaneously downregulating microRNA-9. Infect Immun 85(3). https://doi.org/10.1128/iai.00935-16

Sigler PB, Horwich AL (1995) Unliganded GroEL at 2.8 A: structure and functional implications. Philos Trans R Soc Lond Ser B Biol Sci 348(1323):113–119. https://doi.org/10.1098/rstb.1995.0052

Sigler PB, Xu Z, Rye HS, Burston SG, Fenton WA, Horwich AL (1998) Structure and function in GroEL-mediated protein folding. Annu Rev Biochem 67:581–608. https://doi.org/10.1146/annurev.biochem.67.1.581

Soltys BJ, Gupta RS (1996) Immunoelectron microscopic localization of the 60-kDa heat shock chaperonin protein (Hsp60) in mammalian cells. Exp Cell Res 222(1):16–27. https://doi.org/10.1006/excr.1996.0003

Soltys BJ, Gupta RS (1997) Cell surface localization of the 60 kDa heat shock chaperonin protein (hsp60) in mammalian cells. Cell Biol Int 21(5):315–320. https://doi.org/10.1006/cbir.1997.0144

Sorger PK, Pelham HR (1987) Purification and characterization of a heat-shock element binding protein from yeast. EMBO J 6(10):3035–3041

Speth C, Prohászka Z, Mair M, Stöckl G, Zhu X, Jöbstl B, Füst G, Dierich MP (1999) A 60 kD heat-shock protein-like molecule interacts with the HIV transmembrane glycoprotein gp41. Mol Immunol 36(9):619–628. https://doi.org/10.1016/s0161-5890(99)00082-6

Sreedhar AS, Kalmár E, Csermely P, Shen YF (2004) Hsp90 isoforms: functions, expression and clinical importance. FEBS Lett 562(1–3):11–15. https://doi.org/10.1016/s0014-5793(04)00229-7

Su WY, Gordon T (1997) In vivo exposure to ozone produces an increase in a 72-kDa heat shock protein in guinea pigs. J Appl Physiol 83(3):707–711. https://doi.org/10.1152/jappl.1997.83.3.707

Sun Y, Zheng J, Xu Y, Zhang X (2018) Paraquat-induced inflammatory response of microglia through HSP60/TLR4 signaling. Hum Exp Toxicol 37(11):1161–1168. https://doi.org/10.1177/0960327118758152

Swaroop S, Sengupta N, Suryawanshi AR, Adlakha YK, Basu A (2016) HSP60 plays a regulatory role in IL-1β-induced microglial inflammation via TLR4-p38 MAPK axis. J Neuroinflammation 13:27. https://doi.org/10.1186/s12974-016-0486-x

Swaroop S, Mahadevan A, Shankar SK, Adlakha YK, Basu A (2018) HSP60 critically regulates endogenous IL-1β production in activated microglia by stimulating NLRP3 inflammasome pathway. J Neuroinflammation 15(1):177. https://doi.org/10.1186/s12974-018-1214-5

Swarup V, Das S, Ghosh S, Basu A (2007a) Tumor necrosis factor receptor-1-induced neuronal death by TRADD contributes to the pathogenesis of Japanese encephalitis. J Neurochem 103(2):771–783. https://doi.org/10.1111/j.1471-4159.2007.04790.x

Swarup V, Ghosh J, Duseja R, Ghosh S, Basu A (2007b) Japanese encephalitis virus infection decrease endogenous IL-10 production: correlation with microglial activation and neuronal death. Neurosci Lett 420(2):144–149. https://doi.org/10.1016/j.neulet.2007.04.071

Tanaka Y, Kanai F, Kawakami T, Tateishi K, Ijichi H, Kawabe T, Arakawa Y, Kawakami T, Nishimura T, Shirakata Y, Koike K, Omata M (2004) Interaction of the hepatitis B virus X protein (HBx) with heat shock protein 60 enhances HBx-mediated apoptosis. Biochem Biophys Res Commun 318(2):461–469. https://doi.org/10.1016/j.bbrc.2004.04.046

Tang H, Li J, Liu X, Wang G, Luo M, Deng H (2016) Down-regulation of HSP60 suppresses the proliferation of glioblastoma cells via the ROS/AMPK/mTOR pathway. Sci Rep 6:28388. https://doi.org/10.1038/srep28388

Thounaojam MC, Kaushik DK, Kundu K, Basu A (2014a) MicroRNA-29b modulates Japanese encephalitis virus-induced microglia activation by targeting tumor necrosis factor alpha-induced protein 3. J Neurochem 129(1):143–154. https://doi.org/10.1111/jnc.12609

Thounaojam MC, Kundu K, Kaushik DK, Swaroop S, Mahadevan A, Shankar SK, Basu A (2014b) MicroRNA 155 regulates Japanese encephalitis virus-induced inflammatory response by

targeting Src homology 2-containing inositol phosphatase 1. J Virol 88(9):4798–4810. https://doi.org/10.1128/jvi.02979-13

Thuringer D, Jego G, Wettstein G, Terrier O, Cronier L, Yousfi N, Hébrard S, Bouchot A, Hazoumé A, Joly AL, Gleave M, Rosa-Calatrava M, Solary E, Garrido C (2013) Extracellular HSP27 mediates angiogenesis through Toll-like receptor 3. FASEB J 27(10):4169–4183. https://doi.org/10.1096/fj.12-226977

Tóth ME, Szegedi V, Varga E, Juhász G, Horváth J, Borbély E, Csibrány B, Alföldi R, Lénárt N, Penke B, Sántha M (2013) Overexpression of Hsp27 ameliorates symptoms of Alzheimer's disease in APP/PS1 mice. Cell Stress Chaperones 18(6):759–771. https://doi.org/10.1007/s12192-013-0428-9

Udelsman R, Blake MJ, Stagg CA, Li DG, Putney DJ, Holbrook NJ (1993) Vascular heat shock protein expression in response to stress. Endocrine and autonomic regulation of this age-dependent response. J Clin Invest 91(2):465–473. https://doi.org/10.1172/JCI116224

Vabulas RM, Ahmad-Nejad P, da Costa C, Miethke T, Kirschning CJ, Häcker H, Wagner H (2001) Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. J Biol Chem 276(33):31332–31339. https://doi.org/10.1074/jbc.M103217200

van den IJssel P, Wheelock R, Prescott A, Russell P, Quinlan RA (2003) Nuclear speckle localisation of the small heat shock protein alpha B-crystallin and its inhibition by the R120G cardiomyopathy-linked mutation. Exp Cell Res 287(2):249–261. https://doi.org/10.1016/s0014-4827(03)00092-2

van Eden W, van der Zee R, Prakken B (2005) Heat-shock proteins induce T-cell regulation of chronic inflammation. Nat Rev Immunol 5(4):318–330. https://doi.org/10.1038/nri1593

Veereshwarayya V, Kumar P, Rosen KM, Mestril R, Querfurth HW (2006) Differential effects of mitochondrial heat shock protein 60 and related molecular chaperones to prevent intracellular beta-amyloid-induced inhibition of complex IV and limit apoptosis. J Biol Chem 281(40):29468–29478. https://doi.org/10.1074/jbc.M602533200

Vicart P, Caron A, Guicheney P, Li Z, Prévost MC, Faure A, Chateau D, Chapon F, Tomé F, Dupret JM, Paulin D, Fardeau M (1998) A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet 20(1):92–95. https://doi.org/10.1038/1765

Vilasi S, Carrotta R, Ricci C, Rappa GC, Librizzi F, Martorana V, Ortore MG, Mangione MR (2019) Inhibition of Aβ(1-42) fibrillation by chaperonins: human Hsp60 is a stronger inhibitor than its bacterial homologue GroEL. ACS Chem Neurosci 10(8):3565–3574. https://doi.org/10.1021/acschemneuro.9b00183

von Moltke J, Ayres JS, Kofoed EM, Chavarría-Smith J, Vance RE (2013) Recognition of bacteria by inflammasomes. Annu Rev Immunol 31:73–106. https://doi.org/10.1146/annurev-immunol-032712-095944

Voos W, Röttgers K (2002) Molecular chaperones as essential mediators of mitochondrial biogenesis. Biochim Biophys Acta 1592(1):51–62. https://doi.org/10.1016/s0167-4889(02)00264-1

Wallin RP, Lundqvist A, Moré SH, von Bonin A, Kiessling R, Ljunggren HG (2002) Heat-shock proteins as activators of the innate immune system. Trends Immunol 23(3):130–135. https://doi.org/10.1016/s1471-4906(01)02168-8

Wang CC, Lin WN, Lee CW, Lin CC, Luo SF, Wang JS, Yang CM (2005) Involvement of p42/p44 MAPK, p38 MAPK, JNK, and NF-kappaB in IL-1beta-induced VCAM-1 expression in human tracheal smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 288(2):L227–L237. https://doi.org/10.1152/ajplung.00224.2004

Wang HQ, Xu YX, Zhao XY, Zhao H, Yan J, Sun XB, Guo JC, Zhu CQ (2009) Overexpression of F(0)F(1)-ATP synthase alpha suppresses mutant huntingtin aggregation and toxicity in vitro. Biochem Biophys Res Commun 390(4):1294–1298. https://doi.org/10.1016/j.bbrc.2009.10.139

Wang WY, Tan MS, Yu JT, Tan L (2015) Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med 3(10):136. https://doi.org/10.3978/j.issn.2305-5839.2015.03.49

Wani MA, Mukherjee S, Mallick S, Akbar I, Basu A (2020) Atorvastatin ameliorates viral burden and neural stem/progenitor cell (NSPC) death in an experimental model of Japanese encephalitis. J Biosci 45:77

Weissman JS, Kashi Y, Fenton WA, Horwich AL (1994) GroEL-mediated protein folding proceeds by multiple rounds of binding and release of nonnative forms. Cell 78(4):693–702. https://doi.org/10.1016/0092-8674(94)90533-9

Weissman JS, Hohl CM, Kovalenko O, Kashi Y, Chen S, Braig K, Saibil HR, Fenton WA, Horwich AL (1995) Mechanism of GroEL action: productive release of polypeptide from a sequestered position under GroES. Cell 83(4):577–587. https://doi.org/10.1016/0092-8674(95)90098-5

Weston CR, Davis RJ (2007) The JNK signal transduction pathway. Curr Opin Cell Biol 19(2): 142–149. https://doi.org/10.1016/j.ceb.2007.02.001

Wyss-Coray T, Mucke L (2002) Inflammation in neurodegenerative disease—a double-edged sword. Neuron 35(3):419–432. https://doi.org/10.1016/s0896-6273(02)00794-8

Xu Z, Horwich AL, Sigler PB (1997) The crystal structure of the asymmetric GroEL-GroES-(ADP) 7 chaperonin complex. Nature 388(6644):741–750. https://doi.org/10.1038/41944

Yang Y, Li Z (2005) Roles of heat shock protein gp96 in the ER quality control: redundant or unique function? Mol Cells 20(2):173–182

Yang P, Wang W, Gu H, Li Z, Zhang K, Wang Z, Li R, Duan Y, Zhang S, Wang X (2014) Protection against influenza H7N9 virus challenge with a recombinant NP-M1-HSP60 protein vaccine construct in BALB/c mice. Antivir Res 111:1–7. https://doi.org/10.1016/j.antiviral.2014.08.008

Zanin-Zhorov A, Bruck R, Tal G, Oren S, Aeed H, Hershkoviz R, Cohen IR, Lider O (2005) Heat shock protein 60 inhibits Th1-mediated hepatitis model via innate regulation of Th1/Th2 transcription factors and cytokines. J Immunol 174(6):3227–3236. https://doi.org/10.4049/jimmunol.174.6.3227

Zhou R, Yazdi AS, Menu P, Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. Nature 469(7329):221–225. https://doi.org/10.1038/nature09663

Biphasic Role of Microglia in Healthy and Diseased Brain

Mohit Kumar, Palkin Arora, and Rajat Sandhir

Abstract

Microglia play a very important role in brain development, CNS homeostasis, and response to brain injury and in recovery from neuronal damage. These cells are incredibly plastic in nature with a diversity of phenotype and have the potential to change their functions depending on the brain microenvironment. During neurodegeneration and neuropsychiatric disorders, sterile inflammatory response is induced in the brain through presence of damage-associated inflammatory signals. These include reactive oxygen species, accumulated protein aggregates, endotoxins, and dying cellular debris. Interaction of these signalling molecules with their cognate receptors on microglia can activate immune response in the CNS. Downstream signalling of these molecules further leads to assembly of inflammasome, which induces inflammatory and apoptotic signals in

Mohit Kumar and Palkin Arora contributed equally with all other contributors.

M. Kumar
Department of Biochemistry, Basic Medical Science Block-II, Sector-25, Panjab University, Chandigarh, India

College of Pharmacy, University of Kentucky, Lexington, KY, USA

National Agri-Food Biotechnology Institute, SAS Nagar, Punjab, India

P. Arora
Department of Biochemistry, Basic Medical Science Block-II, Sector-25, Panjab University, Chandigarh, India

Institute of Nuclear Medicine and Allied Sciences (INMAS), DRDO, New Delhi, India

R. Sandhir (✉)
Department of Biochemistry, Basic Medical Science Block-II, Sector-25, Panjab University, Chandigarh, India
e-mail: sandhir@pu.ac.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_18

its environment. Thus, microglial-specific immune activation promotes toxic CNS milieu which in turn affects the phenotypic profile of microglia, further affecting synaptic plasticity, neuronal functions, and behavior phenotype (M1 phenotype). Albeit this, microglia also possess a protective polarization state which releases anti-inflammatory cytokines and reverses neurotoxicity (M2 phenotype). Several studies have opposed the microglial M1/M2 definition, stating heterogeneity in microglial form and function during different stages of the disease. The presence of variable microglial forms and functions poses a challenge for developing therapies and limits our knowledge in terms of disease-specific response of microglia. Therefore, this chapter discusses the recent developments in the understanding of microglial phenotypes along with the role of microglia associated immune pathways in neurological disorders.

Keywords

Major depressive disorder · Microglia · Neurodegeneration · Neuropsychiatric disorder

1 Introduction

First distinguished in 1919 as the third element of the CNS, microglia account for 10–15% of brain cell population. Microglia are resident neuroimmune cells that contribute to neurogenesis (Sato 2015), synaptic pruning (Paolicelli et al. 2011), synaptic plasticity (Tremblay and Majewska 2011), vascular remodeling (Arnold and Betsholtz 2013), behavior response (Lenz and Nelson 2018) and even aid in recover of brain damage during pathophysiological conditions (Colonna and Butovsky 2017). Microglia are involved in three major functions: sensing the environment, phagocytosis of the foreign agents, and housekeeping in the CNS (Hickman et al. 2018) (Fig. 18.1). These plastic cells interconvert between various shapes and forms to sense alterations in the CNS environment. Along with this, a full repertoire of microglial-specific proteins indicated as "sensome" aids in microglial functions during physiological conditions, as discussed in a review article by (Patro et al. 2016). Microglial phagocytic function is attributable to the presence of various cell surface receptors like TLRs, TREM-2, and several other receptors (Patro et al. 2016; Hill et al. 2020). The immunological role of microglia is performed through its capability of chemotaxis, migration, and secretion of inflammatory factors on cellular needs. During brain injury and/or toxic stimulus, activation of the M1 microglia induces production of pro-inflammatory cytokines/chemokines including TNF-α and IL-1β (Lively and Schlichter 2018). Microglia also possesses a protective M2 phenotype that shifts the inflammatory status of the cell into a normal physiological state by releasing anti-inflammatory molecules. Thus, these two polarization states denoted as M1/M2 allow microglia to regulate normal homeostasis in the CNS.

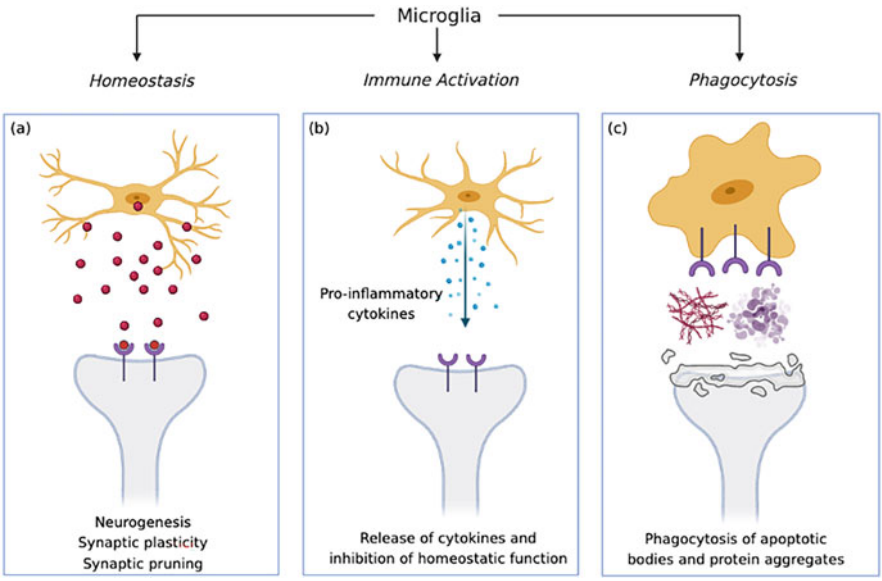

Fig. 18.1 Physiological roles of microglia. The homeostatic microglia is involved in cell-cell communication using a repertoire of ligands and molecules (**a**), which on immune activation gets disrupted due to release of inflammatory mediators (**b**), phagocytic microglia using various receptors can scavenge protein aggregates and apoptotic bodies during degenerative conditions (**c**)

2 The Canonical Bipolar M1/M2 State

The classically activated microglia are denoted as the M1 phenotype with a rounded morphology and expression of CD80, CD86, and CCR7 as cell surface markers. M1 microglia leads to immunological response by an array of immune receptors like TLRs, NODs, and many other scavenger receptors. Conversely, stimulation of microglia with IL-4 cytokine induces the activation of microglia in an alternate phenotype called M2, which produces anti-inflammatory cytokines (IL-4, IL-10, IL-13), enzymes (arginase-1), and growth factors (TGF-β) (Fig. 18.2). These M2 microglia have a typical elongated morphology, which expresses CD163, C-type lectins, CD206, and CD209 as cell surface markers and scavenger receptors (Cherry et al. 2014). In a recent study performed on a rodent model of LPS-induced depression, anxiety, and cognitive deficits, it has been reported that supplementation with a hydrogen sulfide (H_2S; an endogenous gaseous neurotransmitter) donor molecule attenuates LPS-induced behavioral deficits by inhibiting the classical M1 microglia activation, reversing pro-inflammatory cytokine signatures in the brain, and promoting anti-inflammatory M2 microglia type (Kumar and Sandhir 2019; Kumar et al. 2020). Likewise, administration of several other anti-inflammatory

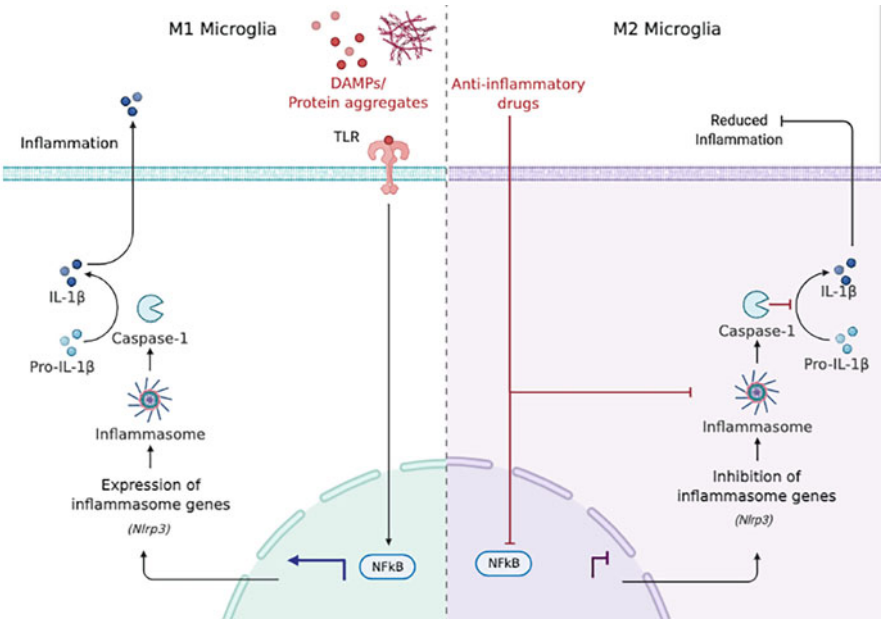

Fig. 18.2 Molecular mechanisms and signaling pathways regulating activation of M1/M2 microglia state

drugs (retinoic acid-loaded polymeric nanoparticles) promotes M2 microglial population and subsequent neuronal survival (Machado-Pereira et al. 2017).

3 Heterogeneity of Microglia

The well-studied canonical bipolar states of microglia, M1 and M2, fail to provide a clear understanding of the various microglia functional profiles. Advancement of the cutting-edge technologies like single-cell RNA-sequencing (scRNAseq), spatial transcriptomics, advanced microscopic techniques like two photon microscopy, and light sheet microscopy are unraveling the morphological, structural, functional, and transcriptional heterogeneity of CNS microglia in regulating brain homeostasis, disease progression, and recovery of damaged neurons in various psychiatric and neurodegenerative diseases.

Under physiological or stress conditions, regional and age-dependent microglial profiles failed to indicate the canonical M1/M2 transcriptome (Grabert et al. 2016). In addition, the animals subjected to traumatic brain injury showed co-expression of M1 and M2 markers using scRNAseq analysis (Kim et al. 2016a). The microglial distribution varies between gray matter and white matter along with variable capacity to self-renewal following LPS insult (Tan et al. 2020). Due to the very plastic and dynamic nature of the microglia, these cells are divided into different subtypes based

on their morphological characteristics like ramified (thin processes, radial branching), primed (thickened processes, lesser secondary branching), reactive (thickened processes with limited branching), and amoeboid (rounded soma with no branching) (Karperien et al. 2013). Recently, microglia have further been categorized to a broad range of subtypes (one to eight) in the adult mouse brains using scRNAseq (Masuda et al. 2020). Further, a regionally heterogenous microglial density is observed in this study corroborating that microglia do not react homogenously toward different toxic stimuli under pathological conditions and may undergo individual changes during the progression of the disease (Lyu et al. 2020).

The microglia also exhibit sexual dimorphism under the influence of early postnatal environment and gonadal hormones. Microglia are sensitive to both estrogen and testosterone that promotes initial sex differences in microglia. Male microglia are reported to have more reactivity with higher migration capacity, whereas female microglia have been shown to have higher phagocytic capacity providing aid and recovery from the brain damage (Yanguas-Casás 2020). Microglia from males exhibit higher immune response with elevated IL-1β mRNA and protein levels following LPS exposure (Loram et al. 2012). Variable anatomical damage coupled with differences in cognitive outcomes is observed between sexes, where ischemic males show significantly larger cognitive deficits than females. Females may possess sex-specific plasticity or compensation due to higher expression of cell repair genes in microglia (Yanguas-Casás 2020).Heterogeneity in microglia thus affects the physiological functions during disease conditions (Fig. 18.3).

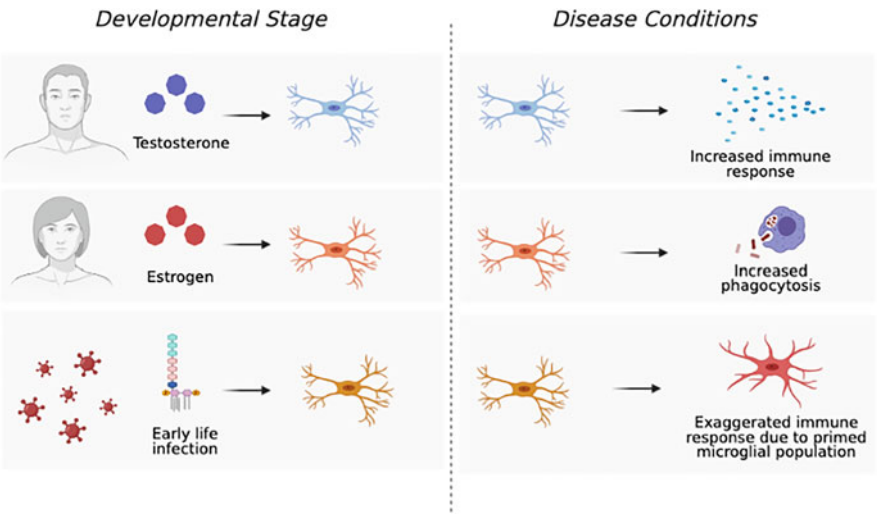

Fig. 18.3 Factors influencing microglial response. The molecules, growth factors, or hormones present in the microenvironment of developing microglia influence their phenotype and behaviour during diseased condition

4 Microglia in Neuropsychiatric Disorders

4.1 Major Depressive Disorder (MDD)

MDD is a complex psychiatric disorder, which includes genetic, cellular, molecular, psychological and social factors (Jesulola et al. 2018). Several mechanisms and theories have been proposed to understand pathogenesis of MDD (Lyu et al. 2020). Despite multifunctional biology of depression, patients relapse occurs on the treatments available, and suffer from the comorbid disorder lifelong. Though recent studies of depression are inclined towards a dysregulated immunological system for a better understanding of clinical depression (Troubat et al. 2021), development of effective therapeutics is of paramount importance. Studies from the clinical data on type 2 diabetes, rheumatoid arthritis, and cancer patients undergoing immunotherapy have shown signs of depression like symptoms. Similarly, systemic administration of endotoxins like LPS to rodents and humans results in depression and anxiety-like behavior (Woelfer et al. 2019; Kumar et al. 2020). Presence of clinical depression in these patients is a consequence of the underlying bidirectional neuron-glia crosstalk in the CNS. This unique interaction is further responsible for alteration in neuronal activity and synaptic plasticity by the release of inflammatory mediators by microglia (Jeon and Kim 2018). An elevation in the peripheral inflammatory cytokines, various acute phase proteins, and circulating innate immune cells have been found in depressed animals along with activation of microglia (Madore et al. 2013). Microglia with their capability to diversify, convert into different morphologies in response to persistent inflammatory milieu show a "hyperactivated phenotype." This phenotypic shift in the microglia can regulate functionality of astrocytes and neurons, hindering their normal physiological state. Many studies have reported an inflammatory shift in microglial phenotype on conditions of neuroinflammatory insult and/or behavioral deficits (Zhang et al. 2018b).

Peripheral immune stimulation with LPS leads to activation of classical M1 microglia and neuroinflammation consequently leading to anxiety and depression in the rodents (Kumar et al. 2020). Pervious study has also reported the presence of DAMPs/PAMPs during stress conditions as the signals for microglial activation via PRR (Pandey et al. 2019). Neuroinflammation during neurological disorders is not induced by the presence of microbial pathogens but induced by the damage-associated inflammatory inducers. DAMPs-associated sterile neuroinflammation induces downstream activation of the NLRP3 inflammasome further activating caspase-1 which subsequently cleaves the pro-IL-1β and pro-IL18 to produce active IL-1β and IL-18 in the brain (Kelley et al. 2019). An elevated level of pro-inflammatory cytokines in the brain shifts the CNS homeostasis to pro-neuroinflammatory state which in turn promotes M1 state of microglia (Song et al. 2017). Activation of microglia instead of dampening the toxic and inflammatory species exaggerates neuroinflammation during depression (Lurie 2018). A significant contribution of NLRP3 inflammasome has been reported, where deficiency of both NLRP3 and its adapter protein ASC attenuated LPS-induced depression-like symptoms. The study also observed increased expression of Indoleamine-

pyrrole 2,3-dioxygenase (IDO) in NLRP3/ASC deficiency (Tang and Le 2016). Along with this, antidepressant treatment has also reported to inhibit activation of inflammasome with a decrease in the levels of serum IL-1β, IL-18, and IL-1β (p17 form). Interestingly, MDD patients without any prior history of treatment showed an increased gene expression of NLRP3 and caspase-1 along with elevated levels of serum IL-1β and IL-18. A 6-week chronic unpredictable mild stress (CUMS) procedure induced the activation of NLRP3 inflammasome, leading to the cleavage of caspase-1, which then resulted in proteolytic processing of IL-1β in the hippocampus (Song et al. 2018). In CUMS-induced rats, administration of apigenin suppressed the production of IL-1β by promoting the expression of PPARγ and inhibiting NLRP3 expression (Li et al. 2016). Moreover, fingolimod (FTY720), a sphingosine analogue, has also been shown to inhibit NLRP3 inflammasome assembly and alleviate CUMS stress-induced behavioral deficits (Guo et al. 2020b). Several studies have highlighted NLRP3 as a potential MDD biomarker as well as a therapeutic target which can be possibly targeted to prevent microglial activation during MDD.

Activation of M1 microglia leads to behavioral deficits such as anxiety, anhedonia, and depression in rodents, and those symptoms were prevented by minocycline pretreatment by inhibiting M1 microglia phenotype (Bassett et al. 2020). Similarly, administration of ketone body metabolite β-hydroxybutyrate (BHB) promoted microglial ramification and M2 polarization both in LPS and chronic unpredictable stress models of MDD (Huang et al. 2018). Treatment with selective serotonin reuptake inhibitors (fluoxetine and S-citalopram) have been shown to downregulate M1 and upregulate M2 microglia markers both in murine BV2 cell line and mouse primary microglia cells (Su et al. 2015). Conclusively, these studies highlight the importance of M2 microglial phenotype in reversing anxiety and depression-like behavior possibly by targeting NLRP3-mediated neuroimmune signaling pathways.

A robust regional heterogeneity in microglia activation has been observed in rodent models of peripheral inflammation-induced behavioral deficits. For example, increased IL-1β response to peripheral *E. coli*-mediated immune challenge was only restricted to the hippocampus of the aging brain (Barrientos et al. 2009). Moreover, LPS exposure showed prominent expression of IL-1β in the cortex than the cerebellum along with reduced expression of cortical cholinergic genes (Silverman et al. 2014). Another study demonstrated that LPS-exposed mesencephalic cultures were more sensitive to neuroinflammation and dopaminergic signaling loss as compared to hippocampal or cortical cultures, indicating a regional susceptibility of neurons to LPS (Kim et al. 2000). Moreover, a regional heterogeneity in microglial and neuroimmune response was also observed in a poly(I:C)-induced maternal immune activation (MIA) mouse model (Garay et al. 2013; Tan et al. 2020). Additionally, meta-analysis of both men and women suffering from MDD showed that women have decreased markers of immune function and microglia as compared to men (Seney et al. 2018). Studies on LPS-induced neuroinflammation showed that partial deletion of BDNF influences the vulnerability to LPS-induced neuroinflammation in a sex-specific manner. A sex-dependent activity of BDNF on neuroinflammation was observed where $BDNF^{+/-}$ females showed increased sensitivity to LPS response

as compared to males (Rossetti et al. 2019). Through recent knowledge of microglial heterogeneity during LPS-induced behavioural deficits, it becomes extremely important to understand region and sex-specific therapeutics to improve treatment of MDD and other neuroinflammation-associated psychiatric disorders.

4.2 Depression and Anxiety

Mental health conditions such as depression and anxiety are associated with psychosocial stress, and understanding their neurobiology is an area of extensive research. Altered bidirectional neuroimmune crosstalk might be plausible biological basis to the stress-related mental health disorders. Similar to MDD, psychosocial stress involves physiological and immunological alterations in the systemic circulation and brain (Kamimura et al. 2020). Experimentally, LPS administration-induced anxiety and depression-like symptoms are used as a model to study psychosocial stress in animals (Van Eeden et al. 2020).

LPS exposure causes neuroinflammation and microglia activation along with increased expression of iNOS, NF-κB p65, NLRP3, ASC, caspase-1, and CD16/32 (M1 microglial markers) leading to depression and anxiety-like behavior (Zhang et al. 2018a). LPS stimulation can prime the microglia to its reactive state promoting a pro-inflammatory microenvironment in the CNS. LPS-induced M1 microglia transforms into a distinctive morphology with reduced branch length and increased soma area. In addition, these structural alterations in microglia led to depressive but anxiolytic behavior in mice which were reversed by the promotion of M2 microglia phenotype using H_2S supplementation (Kumar et al. 2020). Crocin, a natural product has been shown to attenuate depression and anxiety by promoting the expression of CD206, a M2 microglia marker (Zhang et al. 2018a). Neuroinflammation plays a crucial role in the pathophysiology of depression and anxiety. LPS treatment leads to the activation of microglia and production of proinflammatory cytokines in the basolateral amygdala, which plays an integral role in anxiety (Zheng et al. 2021). Administration of BHB has been shown as an antidepressant that inhibits the activation of NLRP3 inflammasome and reduces TNF-α levels in rats exposed to acute and chronic stress (Yamanashi et al. 2017). Additionally, restraint stress (1 h) to mice rapidly increased BHB levels in the blood and NLRP3 in the prefrontal cortex. Restrained mice also showed correlation between depression/anxiety-like behavior with endogenous blood BHB levels (Nishiguchi et al. 2021).

In another model of early life inflammatory stress, expression of NLRP3, ASC, and caspase-1 was increased in the prefrontal cortex and hippocampus with anxiety but not depressive-like behavior (Lei et al. 2017). LPS exposure on postnatal day 14 induces a sexually dimorphic neuroimmune and behavioral response in adulthood (Berkiks et al. 2019a). Early-life immune activation showed a sexually dimorphic behavioral response in rats with both males and females showing anxiety-like behavior, whereas only female rats exhibited depression-like behavior with a concomitant increase in microglia activation, neuroinflammation, and oxidative stress

(Berkiks et al. 2019b). LPS exposure predisposes offspring to amygdala-related disorders such as anxiety and depression with increased expression of Toll-like receptors, pro-inflammatory cytokines, and chronic microgliosis (O'Loughlin et al. 2017). These studies suggest a crucial role of microglial polarization in cognitive and emotional well-being, which can further be affected by other factors like sexual dimorphism and early life infection.

4.3 Bipolar Disorder

Microglia from bipolar patients show elevated microglial density and microglial-specific markers in the medial frontal gyrus in comparison to the superior temporal gyrus and thalamus. LPS stimulation to microglia of these regions showed absence of immune activation (Sneeboer et al. 2019). On the contrary, immune involvement of microglia has been found during bipolar disorder (BD) wherein downregulation of CD206 (M2 microglial marker) is positively correlated with manic state (Ohgidani et al. 2017). Further, understanding the inflammatory trend during BD has shown increase in both neuroinflammatory and systemic inflammatory processes. Higher levels of monocyte chemoattractant protein-1 (MCP-1), YKL-40 (also known as chitinase 3-like 1), sCD14, and tissue inhibitor matrix metalloproteinase-1 and 2 (TIMP-1 and TIMP-2) in the CSF along with elevated serum sCD14 and YKL-40 levels have been observed in BD patients (Jakobsson et al. 2015). Studies have also reported the association between mitochondrial dysfunction and neuroinflammation as one of the key contributors to the pathogenesis and progression of the BD. Neuroinflammation induced by elevated ROS levels due to the decreased levels of mitochondrial complex I subunits observed in BD patients might be responsible for behavioral phenotypes in BD (Kim et al. 2015). Findings from clinical studies performed on postmortem brain samples from BD patients further support complex I dysfunction along with NLRP3 inflammasome activation in BD (Kim et al. 2016b). Interestingly, patients with BD had upregulation of TSPO pathway proteins (TSPO and VDAC), NLRP3, ASC, and pro-caspase1 followed by an increase in caspase-1 activity, IL-1β, and IL-18 levels along with accumulation of dysfunctional mitochondria (Scaini et al. 2019). The presence of M1 microglial-associated proinflammatory cytokines and chemokines along with mitochondrial dysfunction indicates a potential role of microglia in the pathogenesis of BD (Sakrajda and Szczepankiewicz 2021).

The role of microglia and inflammation during BD suffers from several contrasting data which in turn hinders our understanding and challenges development of novel therapeutics. Studies have found a nonsignificant change in the mean estimated density of Iba1-immunostained microglial cells in the anterior midcingulate cortex (aMCC) in postmortem brains along with a negative association between CSF YKL-40 and prospective manic/hypomanic episodes (Isgren et al. 2017). Another study on fractalkine signaling, important for microglia-neuron crosstalk, reported downregulation of CX3CR1 in gray and white matter in BD postmortem brain, but no change in fractalkine (CX3C ligand) levels was found in

the frontal cortex (Sneeboer et al. 2019; Hill et al. 2020). These difference in microglial activation and immune function could possibly be a consequence of regional diversity of microglia.

4.4 Schizophrenia

Schizophrenia (SCZ) is a serious mental illness with social impairment and chronic neurodegenerative characteristics (Tomasik et al. 2016). Uncontrolled activity of pro-inflammatory cytokines and microglial activation in tandem with genetic vulnerability and glutamatergic neurotransmitter toxicity are considered as the underlying pathological mechanisms (Na et al. 2014). Clinical studies analyzing patients' blood and CSF, neuroimaging, and postmortem brain tissue suggest that aberrant immune responses through altered neuroplasticity defines SCZ (De Picker et al. 2017). In vivo microglial PET imaging studies have shown moderate elevations in TSPO tracer binding in gray matter relative to other brain regions (Marques et al. 2019). According to the microglia hypothesis of SCZ, immunological activators like LPS/IFN-γ induced by stressful life events are responsible for activation of M1 microglia and subsequent release of pro-inflammatory cytokines and free radicals. In turn the activated microglia disrupt the microglia-neuron interaction leading to neuronal degeneration, white matter abnormalities, and decreased neurogenesis in SCZ (Monji et al. 2009).

Meta-analysis of studies on SCZ has found increased IL-6 and IL-8 levels in the CSF of the affected individuals (Orlovska-Waast et al. 2019). Along with this, increasing evidence shows association between oxidative stress and inflammation during SCZ (Sawa and Sedlak 2016). A recent clinical study on postmortem brain of multiple psychiatric diseases including SCZ has found transcriptional changes along with increased neuroinflammation in dorsolateral prefrontal cortex, dorsal striatum, and hippocampus (Lanz et al. 2019). A clinical trial has reported reduced IL-1β levels in patients' CSF and serum along with decreased soluble IL-2 receptor levels in CSF but high IL-2R in serum, therefore suggesting neuroimmunological abnormalities in the SCZ (Barak et al. 1995). Additionally, it has been found that sIL-6R levels have a positive correlation with the development of SCZ in patients predisposed to early life infection (Hartwig et al. 2017). On similar lines, prenatal or neonatal challenge with poly (I:C), a TLR-3 agonist, is widely accepted as a neurodevelopmental animal model of SCZ (Karlsson and Dalman 2020). It has been found that poly(I:C) induction leads to a significant increase in iNOS levels in the hippocampus, cortex, and corpus callosum of poly(I:C)-affected offsprings. Along with these, circulating levels of pro-inflammatory cytokines (TNF-α and IL-6) are elevated in offsprings, thus confirming influence of maternal immune activation on neurobiology of offspring (Esshili et al. 2020).

Interestingly, microglial activation not only regulates neuroinflammatory processes but is also involved in other physiological responses in the brain. As in a case of SCZ, reduced synaptic density is observed in postmortem cortical region (Sellgren et al. 2019). This reduction in synapse density is reflective of abnormality

in microglia-associated synaptic pruning, which on minocycline administration attenuates synaptic loss and decreases risk of SCZ. This decrease in synaptic density in cortex possibly could be due to activated microglial-associated metabolic alterations which impair synapse formation and neurotransmission (Park et al. 2020).

Overall, studies on SCZ postulate an important role of microglial activation and direct toward inhibition of this glial state with promotion of protective phenotype. Studies have always highlighted the immunological perspective of microglia activation, it is also crucial to highlight the consequences of decline in homeostatic functions of microglia. Pharmacological studies have reported inhibition of microglial activation using minocycline in both prevention of synapse loss and inflammatory insult. Further studies are still required to better understand the phenotypic diversity of microglia throughout the brain and to open a new avenue for potential pharmacological interventions in SCZ.

5 Microglia in Neurodegenerative Diseases

Microglia express receptors for neurons-derived biomolecules like neurotransmitters and neuropeptides which enable neuron-glial crosstalk (Colonna and Butovsky 2017). These receptors direct microglial processes towards the neurons and help to regulate the synaptic plasticity and dendritic spine density, and any physiological damage to neurons triggers a cascade of signals to microglia for phagocytoses of damaged neurons and removal of debris (Tian et al. 2012). Several immunoglobulin superfamily (IgSF) members present on both neurons and microglia also help to downregulate the inflammatory response (Tian et al. 2012), whereas during degenerative conditions, presence of protein aggregates and other toxic species leads to microglial shift activated state which further promotes neuroinflammatory conditions that enhance cytotoxicity. Unfortunately, dying neurons act as a chronic signal for microglia contributing to progressive neuronal loss. However, during neurodegeneration microglia-neuron crosstalk is disrupted due to prolonged microglial activation state. In turn, absence of this communication alters the housekeeping function of microglia and impairs neuronal survival (Marinelli et al. 2019). Thus, presence of endogenous toxic species and activation of microglia and inflammatory pathways including NLRP3 inflammasome impair microglial physiology and henceforth neuronal survival leading to neurodegeneration and promote progression of various neurodegenerative diseases as discussed below:

6 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease and is the most common cause of dementia in the elderly worldwide. It is characterized by brain atrophy, amyloid plaques, neurofibrillary tangles, loss of neurons and synapses, dystrophic neuritis, microgliosis, and astrogliosis (Stelzmann et al. 1995). It is a heterogeneous disorder caused by underlying genetic and environmental factors,

where AD-specific risk genes have been found as the major contributors in disease pathology. In the last decade around 20 novel genetic loci have been identified using genome wide associated study (GWAS) strongly associated with the high risk of AD, where APOE gene has been reported as the major contributor in pathogenesis and progression of AD (Bertram and Tanzi 2009). In addition, rare genetic variants associated with AD have also implicated an important role of microglia in development of AD. Along with this, the most important discovery was of the AD-associated variants in TREM2 (Hansen et al. 2018). Additionally, an association between polymorphism in the interleukin-1 receptor accessory protein (IL1RAP) gene and Aβ accumulation in human brains has further pointed towards the role of microglia in AD (Ramanan et al. 2015).

The presence of senile plaques, intracellular aggregates of hyperphosphorylated tau, and extensive neuronal loss triggers neuroinflammatory response by activating NLRP3 inflammasome in the microglia (Zhang et al. 2020b). The senile plaques and phosphorylated tau prime TLR on microglia which activate NLRP3 inflammasome. Presence of ATP, K^+ efflux, and pore-forming toxins can also activate NLRP3 inflammasomes leading to detrimental effects on neurons (Pereira et al. 2019). Studies have shown that loss of NLRP3 inflammasome attenuated tau hyperphosphorylation and aggregation by regulating tau kinases and phosphatases during AD (Heneka et al. 2013; Ising et al. 2019). Perhaps, microglia-targeted pharmacological inhibition of NLRP3-mediated neuroimmune response during AD may help to prevent pathophysiology of AD and associated behavioral deficits in aged individuals.

Recent ultrastructural analysis of mice and postmortem human brain-resident microglia in AD have shown diverse population of microglial cells during amyloid presence (El Hajj et al. 2019). According to recent study (Holtman et al. 2017), the possible molecular mechanisms underlining microglial heterogeneity in AD brain involving (1) Signal-dependent transcriptional factors (SDTF) for meditating inflammation, (2) Lineage determining transcription factors (LDTF) for maintaining a healthy surveillance, and (3) Transcription factors essential for survival and differentiation. Any imbalance in any of these regulating factors may result in perturbed microglial function and exacerbation of the underlying AD pathology. To date, five microglial clusters have been identified in AD brain: plaque-associated microglia (PAM), disease-associated microglia (DAM), human Alzheimer's microglia (HAM), dark microglia, and subcluster C9 in FNX (Hashemiaghdam and Mroczek 2020). These microglial subtypes have a characteristic gene expression profiles and are present during different stages of the disease progression, thus pointing towards microglial heterogeneity as a new area for exploration in AD research. Out of these subclusters of the microglia, DAMs are associated with TREM2, an important mediator for the onset and progression of AD. As mentioned earlier, TREM2 variants are found to be involved in the pathology of AD, and it has also been reported that TREM2 leads to the activation of DAM (Hashemiaghdam and Mroczek 2020). Homozygous and heterozygous loss of TREM2 results in impaired phagocytotic activity of microglia, which in turn affects Aβ clearance as TREM2 binds directly to lipoproteins and facilitates Aβ-lipoprotein uptake by microglia

(Claes et al. 2019). Changes in CSF sTREM2 occur only after alterations in the brain amyloidosis and neuronal injury, and temporal quantification of sTREM2 can act as a potential early biomarker of AD. sTREM2 levels can also help in understanding the drug efficacy, which potentiates it as a biomarker for therapeutic purposes as well (Suárez-Calvet et al. 2016). Thus, a diverse population of microglia leads to activation of neuroimmune pathways both in central and peripheral nervous systems during the progression of AD.

7 Parkinson's Disease

Parkinson's disease (PD) is characterized by motor dysfunction including bradykinesia, muscle rigidity, and resting tremors. Along with motor abnormalities, patients also show signs of neuropsychiatric, autonomic, sleep, and sensory deficits (DeMaagd and Philip 2015). The major neuropathological manifestations of PD include α-synuclein-containing Lewy bodies and loss of dopaminergic neurons in the substantia nigra (Mehra et al. 2019). L-DOPA is considered as the mainstay of treatment for PD patients, while several advance therapies and deep brain stimulation can also be used for the management of PD (Balestrino and Schapira 2020). α-synuclein (α-Syn) plays a critical role in progression of PD by influencing microglial population and neuroinflammatory response (Ferreira and Romero-Ramos 2018). PD risk factors such as DJ-1, LRRK2, and α-Syn serve as a molecular link between microglia-mediated neuroinflammation and pathophysiology of PD (Moehle et al. 2012). α-Syn acts as a major contributor in the pathogenesis of PD in several ways: Induces activation of M1 microglial population. Regulates microglial phagocytosis, and Affects microglial activation and inflammatory response by interacting with various receptors on microglia surface (Ho 2019). Contribution of microglia in the development of PD remains an intensive area of the research, where GWAS have identified the PD-associated risk genes like human leukocyte antigen gene (HLA-DRA) and TREM-2 that are expressed specifically on microglia (Hamza et al. 2010; Han et al. 2017). The deficiency of TREM2 inhibits M2 polarization and promotes M1 microglial inflammatory responses, wherein overexpression of TREM2 alleviates inflammatory response and promotes M2 microglial phenotype (Zhang et al. 2018c). In addition, activation of microglia promotes cell apoptosis in dopaminergic (DA) neurons and thus plays an essential role in the progression of PD (Jiang et al. 2019).

The degeneration of DA neurons is a hallmark of PD pathology, and LPS-induced activation of microglia in rodents has also reported a microglia-dependent loss of DA neurons in the brain regions associated with PD (Chien et al. 2016). The molecular mechanisms underlying DA neuronal loss is accumulation of α-synuclein which acts as a DAMP for TLR1/2, leading to activation of downstream signaling mediators and release of pro-inflammatory cytokines (Béraud et al. 2011). As in case of other neurodegenerative conditions, activation of M1 microglia leads to a neuroinflammatory microenvironment in PD (Babcock et al. 2006). In addition, PD patients also show an elevation in pro-inflammatory mediators such as TNFα,

IL-1β, and IL-6 in the CSF and striatum. The elevated cytokines start early, accompany neurodegeneration, and persist throughout the course of PD (Nagatsu et al. 2000).

A loop of events occur during onset of PD pathology, where α-syn-stimulated microglia induces NLRP3 inflammasome-dependent degeneration in DA neurons, and in turn NLRP3 inflammasome is responsible in promoting aggregation of α-syn. Usage of NLRP3 inhibitor MCC950 prevents syn pathology and DA neurodegeneration in mice (Gordon et al. 2018). The basic mechanism behind NLRP3 inflammasome involves exosomes that are released from microglia under prolonged manganese exposure (Sarkar et al. 2019). During PD these exosomes have been found to carry significantly higher levels of NLRP3 inflammasome-associated proteins (ASC, caspase-1) and have an increased intercellular transport activity (Guo et al. 2020a). In addition, using radioligand targeting TSPO, [^{18}F]-DPA714, a specific topographic pattern of microglial activation, is reported with increased number of activated microglia in the nigro-striatal pathway and the frontal cortex of PD patients (Lavisse et al. 2021). Interestingly, a study has found preferential activation of microglia during PD and showed that microgliosis accompanied α-syn aggregation (Kim et al. 2020), therefore indicating an important role of microglia in the progression and development of PD.

8 Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by defective huntingtin gene with CAG trinucleotide repeats (Walker 2007). It is characterized by neurobehavioral defects including movement and cognitive deficits. The presence of mutant huntingtin (mHtt) in gamma-aminobutyric acid (GABA)ergic striatal medium spiny neurons show early signs of neurodegeneration in the striatum which then progresses to the cortical region. A mounting evidence of microglia activation in the postmortem brains from presymptomatic HD carriers has been established (Yang et al. 2017). Along with this an elevation in pro-inflammatory cytokine signatures are observed in both the CNS and plasma of HD patients (Crotti and Glass 2015).

The localization of thymosin beta-4 (Tbeta4), a reactive microglial marker, shows an early and proximate association of activated microglia in the neostriatum, cortex, and globus pallidus and adjoining white matter in HD brains (Sapp et al. 2001). Another PET marker of microglial activation, [(11)C](R)-PK11195, supports the contribution of microglia in inducing neurodegeneration in striatum of HD patients (Pavese et al. 2006). In addition, this PET ligand demonstrates microglial activation as an early event of HD pathogenesis, and use of this marker can help in improving efficacy of neuroprotection strategies (Tai et al. 2007). A study compared the presence of nuclear inclusions of mutant huntingtin in neurons and glial cells in the brain (Jansen et al. 2017). The clinical and preclinical models of HD showed differences in cellular localization of the mHtt nuclear inclusions, with their absence in rodent microglia (Rodrigues et al. 2016). The study highlighted an important

aspect of research where translational studies are required to understand the cellular and biochemical changes observed both in clinical and preclinical HD models (Jansen et al. 2017). In the vicinity of neurons expressing mHtt fragments, microglial proliferation and inflammatory signals help in removal of dysfunctional neurites or aberrant synapses improving the neuronal functions (Kraft et al. 2012). With the increased accumulation of mHtt result in primed microglia leading to a secondary injury further exaggerating the inflammatory response (Perry and Holmes 2014). In support to this, microglial population in HD shows dominance of microglia with M1 phenotype which is followed by release of pro-inflammatory cytokines in the CNS (Björkqvist et al. 2008). Along with immune activation in the CNS, HD patients also show signs of systemic abnormalities, wherein presence of mHtt in monocytes and an altered immune profile in the plasma of HD patients has been reported. The same study has also suggested an important role of peripheral inflammatory response in revealing early pathogenic events in HD.

The M1 microglia play a crucial role in the pathogenesis of HD, as M1-associated biomarkers are detected in R6/2 transgenic mouse model of HD (Benraiss et al. 2016). Along with increased levels of iNOS, elevated levels of IL-1β, IL-6, and TNF-α were also observed after LPS treatment in R6/2 mouse brain. Elevated levels of IL-1β and IL-8 were also observed in a transgenic porcine model of HD (Valekova et al. 2016). Furthermore, an increase in the pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) were also observed in the brain, CSF and plasma of HD patients (Yang et al. 2017). Interestingly, transformation of microglia to M2 state are found to augment the anti-inflammatory effects in the presence of IL-4 and with increased levels of Arg1 and switching of microglial state from M1 to M2 demonstrates protective potential during HD (Tang 2018). A study also reports co-existence of IL-10, VEGF, TGF-β, and IGF-1 with M1 markers in postmortem HD brains. But studies on microglial heterogeneity are still limited in HD (Chang et al. 2015).

Moreover, a caspase-1-dependent cell death, known as pyroptosis, is linked to the inflammasome activity (Miao et al. 2011). Studies have found occurrence of pyroptotic cell death in HD mice. Administration of olaparib, an antineoplastic compound, acts as an inhibitor of cell death resulting in decline in pyroptosis (Paldino et al. 2020a). Co-expression of NLRP3 inflammasome with pyroptosis has been observed in striatal spiny projection neurons and in parvalbumin interneurons of HD mice (Paldino et al. 2020b). Thus, HD involves an important role of reactive microglia along with activation of inflammatory pathways.

9 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting motor neurons in the brain stem, spinal cord, and primary motor cortex (Wijesekera and Leigh 2009). Neuroinflammation-induced motor neuron degeneration, wherein activation of astrocytes and microglia leads to neurodegeneration and determination of the disease phenotype by their local inflammatory response in ALS

(Ilieva et al. 2009). Alterations in several other cell types including muscle fibers and oligodendrocytes along with astrocytes and microglia is observed as early properties of ALS (Rossi et al. 2016).

ALS can be sporadic or familial, and familial ALS contributes only 5–10% of the affected population. Mutation in several genes has been reported as the underlying cause of familial ALS which includes superoxide dismutase *1* (*SOD1*), fused in sarcoma/translocated in liposarcoma *(FUS/TLS),* transactive response DNA-binding protein-43 *(TDP-43),* TANK-binding kinase *1 (*TBK1), and chromosome 9 open reading frame 72 (C9orf72).

A hexanucleotide repeat (GGGGCC) expansion in an intron of the *C9orf72* gene generates toxic RNA species as well as harmful dipeptide-repeats formation (Balendra and Isaacs 2018). A decreased expression of *C9orf72* observed in expansion carriers leads to lysosomal accumulation and altered immune responses in macrophage and microglia, thus confirming that C9orf72 is required for both microglial and myeloid function (O'Rourke et al. 2016). In addition, ALS-susceptibility gene, TBK1, which is involved in autophagy processes, also regulates the production of interferon (IFN-α) and IFN-β confirming that impaired autophagy along with release of pro-inflammatory cytokines can be a mediator for microglial activation during ALS (Geloso et al. 2017). Mutant forms of *TDP-43*, neuroinflammation with activated microglia, activation of NF-κB and AP-1 pathways, and activation of NLRP3 inflammasomes are observed as pathological mechanisms observed during ALS (Zhao et al. 2015). Similarly, mutant mSOD1 (G93A) or mSOD1(G85R) induces activation of microglia with a concomitant release of pro-inflammatory cytokines and ROS further leading to motor neuron degeneration (Zhao et al. 2010). Therefore, these studies signify a direct relation between microglia and ALS-associated mutant genes: *SOD1*, *FUS/TLS, TDP-43,* TBK1, and *C9orf72.*

Physiologically, cellular stress during ALS elevates c-Ret gene expression in microglia but not in motor neurons. Microglial c-Ret interacts with glia-derived neurotrophic factor (GDNF) and improves microglial survival in ALS, whereas deprivation of GDNF in the spinal cord by activated microglia may lead to neuronal damage (Lee et al. 2016). Hierarchical cluster analysis of microglia has shown a heterogenous population of microglia with diverse shape, morphology, and phenotype in the ventral horn of the lumbar spinal cord of SOD1(G93A) transgenic mice (Ohgomori et al. 2016). Along with these morphometric changes, microglia exhibited M1 state markers in the early stage of the disease which was followed by expression of M2 microglia markers during the slowly progressing phase. Pharmacological interventions that promote M2 microglia polarization can be beneficial in diminishing the neurotoxic effects of the M1 state. Furthermore, aggregated SOD1 (G93A) activates microglial NLRP3 inflammasome leading to secretion of IL-1β cytokine in a dose- and time-dependent manner (Deora et al. 2020). Mutant form of TDP-43 protein also activates microglial inflammasomes in an NLRP3-dependent manner (Deora et al. 2020). Use of anti-inflammatory drug cyclo (His-Pro), led to the inhibition of NLRP3 inflammasome along with reduction in protein nitration and a decline in NO and ROS levels in ALS-induced microglial

cells (Grottelli et al. 2019). In addition, it was found that an increase in TLR4 and nuclear NF-κB levels primed the NLRP3 inflammasome activation in transgenic mouse model of ALS (Gugliandolo et al. 2018). The associated increase in IL-1β, IL-18, and IFN-γ amount was also observed in the spleen of transgenic rats, together with an increased expression of CD4, CD8, CD44, and CD68 markers indicated an important role of central as well as peripheral immune system during ALS (Gugliandolo et al. 2018). Studies have also found expression of NLRP3 inflammasome, ASC, and IL-1β in the neurons suggesting their contribution in neurodegeneration in the anterior thalamus, which might be a reason for behavioral deficits observed during ALS pathology (Debye et al. 2018).

During early stages of the disease, neuronal loss and neuroinflammation are regionally distinct (Lewis et al. 2012). Some CNS regions are severely affected, while other regions show compensatory mechanisms where neuroprotective gliosis preserves the neuronal functions. Microglial homeostatic gene Sal-like1 (Sall1) is increased in the white matter of control and presymptomatic ALS animals, whereas it loses its homeostatic function and shows a pro-inflammatory phenotype at the end stage of ALS (Maniatis et al. 2019). Furthermore, Trem2/DAP12 signaling is identified as key switch to regulate microglial phenotype from a homeostatic to a DAM state during the progression of ALS. A regional pattern of motor neuron loss during ALS shows heterogeneity in the local environment and glial activation, thus highlighting a challenge for pharmacological interventions which would turn out to be futile in a heterogenous functional state of the CNS (Cipollina et al. 2020).

10 Multiple Sclerosis

Multiple sclerosis (MS) is a common chronic inflammatory disease of the CNS, triggered by autoreactive lymphocytes reacting against CNS autoantigens. Compromised blood brain barrier and infiltration of the peripheral immune cells into the brain parenchyma are the hallmark of MS. Neuroinflammation induced by reactive microglia and astroglia promotes demyelination and neurodegeneration in MS patients (Luo et al. 2017; Ponath et al. 2018). Experimental autoimmune encephalomyelitis (EAE) and cuprizone-induced demyelination are the most commonly used experimental models to study MS (Bjelobaba et al. 2017).

Neuroinflammation and reactive gliosis during MS are not limited to the demyelinating lesion area but are also pronounced in intact white matter regions. Reactive microglia acquire phagocytotic nature at the edge of the active lesions, suggesting an important role of microglia in plaque formation and tissue damage in MS patients (O'Loughlin et al. 2018). Activated microglia-induced neuroinflammation plays a central in the pathogenesis of MS (Voet et al. 2019). Loss of myelin sheath is a pathological phenomenon of MS accompanied by loss of myelin-forming cells, oligodendrocytes (OLs), and a decline in function of oligodendrocyte precursor cells (OPCs). OPCs are widely distributed throughout the adult CNS and with their capacity to proliferate, migrate, and differentiate into mature OLs help in the process of remyelination. Inadequate myelin debris clearance and presence of

inflammatory and reactive species inhibit the recruitment and proliferation of OPCs. Thus, microglia-induced oxidative stress and pro-inflammatory environment lead to demyelination as well as inhibition of remyelination during MS (Luo et al. 2017).

Microglia also play an important role in infiltration of adaptive immune cells into the brain parenchyma during the progression of MS. Activated microglia act as antigen-presenting cells, expressing class I and II MHCs interacting with CD28 or cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) on T cells, while the costimulatory molecules B7–1 and B7–2 bind to CD28 resulting in T cell proliferation and differentiation (Sansom 2000). Crosstalk between microglia and T cells results in different outcomes depending upon variable environmental cues. It has been found that myelin basic protein (MBP)-primed T cells induce contact-mediated expression of iNOS in microglial cells (Dasgupta et al. 2002), whereas Th2 polarization of MBP-primed T cells resulted in expression of neurotrophic molecules such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) (Roy et al. 2007). Similarly, it has been found that MHC class II(+)CD40(dim)CD86(dim)IL-10(+) microglia are potent inducers of Ag-specific CD4(+)Foxp3(+) regulatory T cells (Tregs) in vitro (Ebner et al. 2013), thus indicating that microglia have regulatory properties on T cell activation profile which can be further explored for drug designing.

Interestingly, it has been observed that internalization of myelin by microglia leads to the expression of Arg-1, an enzyme that converts arginine to ornithine with release of NO. Myelin acts as an antigenic stimulus that shifts the microglia population into its reactive M1 phenotype which is responsible for releasing inflammatory cytokines, chemokines, reactive species, and NO in the CNS milieu. Along with M1 phenotypic shift, microglial-induced NLPR3 inflammasome activation is reported during MS (Guerrero and Sicotte 2020). Activation of the NLRP3 inflammasome has also been shown to be associated with the pathogenesis and progression of MS, where increased expression of NLRP3 inflammasome elements has been observed in microglia and other neuroimmune cells. On exposure to inflammatory stimuli, human microglia and oligodendrocytes also showed inflammasome activation and pyroptosis in vitro (McKenzie et al. 2018). Moreover, an elevated level of NLRP3 inflammasome was also observed in monocytes isolated from the patients with primary progressive MS (Malhotra et al. 2020). Altogether, these results demonstrate IL1β and NLRP3 as a prognostic biomarker and microglia as potential therapeutic target for the management of MS.

Attenuation of M1 phenotype with a shift toward M2 polarization using progesterone therapy has been found to be beneficial in cuprizone-induced demyelination mouse model. Along with this, progesterone therapy has also been shown to decrease NLRP3 inflammasome and IL-18 expression with a concomitant decline in demyelination (Aryanpour et al. 2017). Exosomes secreted by the bone marrow mesenchymal stem cells also resulted in a significant increase in the levels of M2-related cytokines such as IL-10 and TGF-β, whereas M1-related TNF-α and IL-12 levels were decreased significantly (Li et al. 2019). A regional heterogeneity is further observed in the brain of rodent model of cuprizone-induced demyelination, where a significant increase in the number of activated microglia was observed in the

cortex as compared to the corpus callosum (Gudi et al. 2009). A high molecular heterogeneity was also observed between MS lesion types, where seven different subtypes of microglia have been identified which are distinguished as homeostatic (*P2RY12*, *RUNX1*, *CSF1R*), MS-specific (microglia activation markers, complement molecules, and *MHC-II*, *ASAH1*, *ACSL1*, *DPYD* genes), and phagocytosis- and oligodendrocytes-associated genes (*CD163*, *PLP1*, *MBP*, and *ST18*) (Schirmer et al. 2019). These studies suggest the robust regional heterogeneity in microglia in MS tissues. Future studies are warranted to further analyze the different MS brain lesion types and associated microglia subtypes.

11 Clinical Significance

The microglia from human and mice are quite similar. In spite of the similarities, there are many interspecies differences thus emphasize need for extensive research. Most of the common neurodegenerative disorders show similar pathophysiological-mechanisms wherein activation of microglia results into a sequalae of neuroinflammatory processes with similar patterns of activation in both humans and animal models (Sheeler et al. 2020). Immunological therapy, a recent advancement in targeting neurodegeneration, failed to show beneficial results as clinical trials of drugs such as pioglitazone, a peroxisome-proliferator activated receptor γ (PPARγ) agonist for AD, were terminated at phase III trials (Dong et al. 2019). Similarly, use of non steroidal anti inflammatory drugs (NSAIDs) in patients with neurodegenerative diseases showed lower efficacy and higher side effects, while replacing natural products and derivates may be a possible therapeutic strategy (Jin et al. 2019).

On the other hand, research on neuropsychiatric disorders is in infancy and requires extensive clinical research to determine the microglial activation and phenotype investigation. Some human centered studies show confounding results with absence of microglial activation accompanied by increased concentration of cytokine levels in the circulation during psychological impairment (Suzuki et al. 2019). Therefore, it is becomes crucial at the level of both disease identification and drug development to understand microglial activation as an important perspective with a focus on factors like bipolarity of microglia and mechanisms for M1/M2 switching.

12 Conclusions

Microglia are major contributors in the development and progression of neurodegeneration and psychiatric disorders. The plastic nature of these cells help them to transform into different states (M1/M2) and respond according to the environmental cues, whereas microglial functions are hindered during neurodegeneration due to accumulation of cellular debris and toxic aggregates resulting in a hyperactivated microglial state. Similarly, during psychiatric

Table 18.1 Drugs targeting microglial activation along with the M1/M2 phenotypic switching during neuropsychiatric disorders

Disease	Model	Drug	Reference
MDD	LPS-induced depression	Melatonin	Arioz et al. (2019)
MDD	LPS-stimulated microglial culture	Tianeptine	Ślusarczyk et al. (2018)
MDD	LPS-induced depression	Clomipramine	Gong et al. (2019)
MDD	CUMS	Minocycline	Zhang et al. (2019a)
MDD	CUMS	Fingolimod	Guo et al. (2020b)
MDD/ depression	LPS-stimulated microglial culture	Fluoxetine and S-citalopram	Su et al. (2015)
Depression/ anxiety	LPS-induced depression	Crocin	Zhang et al. (2018a)
Depression/ anxiety	LPS-induced depression	Melatonin	Berkiks et al. (2018)
Depression/ anxiety	LPS-induced depression	Hydrogen sulfide (H_2S)	Kumar et al. (2020)
Bipolar disorder	D-amphetamine mania model	Doxycycline	Chaves Filho et al. (2021)
Schizophrenia	Schizophrenia patients	Minocycline	Zhang et al. (2019b)

Table 18.2 Drugs targeting microglial activation along with the M1/M2 phenotypic switching during neurodegenerative disorders

Disease	Model	Drug	Reference
AD	Aβ1-42-induced microglia activation	Naringenin	Yang et al. (2019)
AD	APP/PS1 transgenic AD mice	TAK-242 (TLR4-specific inhibitor)	Cui et al. (2020)
AD	APP/PS1 transgenic mice	Liquiritigenin	Du et al. (2020)
AD	APP/PS1 transgenic mice	Deferoxamine	Zhang and He (2017)
PD	MPTP model of PD	Idebenone	Yan et al. (2019)
PD	LPS-induced lesion in substantia nigra	Capsaicin	Bok et al. (2018)
PD	MPTP model of PD	Vitamin D	Calvello et al. (2017)
PD	LPS and α-synuclein	Kaempferol	Han et al. (2019)
PD	MitoPark/6-OHDA/α-synuclein	MCC950	Gordon et al. (2018)
PD	LPS/MPP+	Andrographolide	Ahmed et al. (2021)
PD	MPP+	Donepezil	Chen et al. (2015)
MS	CPZ model	Progesterone	Aryanpour et al. (2017)

(continued)

Table 18.2 (continued)

Disease	Model	Drug	Reference
MS	EAE rat model	Mesenchymal stem (MSCs) cell-derived exosomes	Li et al. (2019)
MS	EAE model	Pleiotrophin	Miao et al. (2019)
MS	CPZ model	Mesenchymal stem cell	Barati et al. (2019)
MS	EAE model	Tetramethylpyrazine	Zhang et al. (2020a)
ALS	SOD(1G93A) mouse model	Rofecoxib	Zou et al. (2020)
ALS	SOD(1G93A) mouse model	Clemastine	Apolloni et al. (2016)
HD	R6/2 HD transgenic mice	Mesenchymal stem cells (MSCs)	Yu-Taeger et al. (2019)
HD	3-NP-induced HD	Quercetin	Chakraborty et al. (2014)
HD	Primary microglia from R6/2 mice	Suberoylanilide hydroxamic acid (SAHA)	Giorgini et al. (2008)
HD	Quinolinic acid model	Aminoguanidine	Ryu et al. (2004)

disturbances, microglia present an activated phenotype which can alter neurological functions and behavioral phenotype. Studies devoted to understanding microglia during disease conditions baffle between heterogeneity of microglial forms and functions which are further influenced by sexual dimorphism and early life experience. According to the canonical definition of bipolarity, drugs inhibiting M1 and promoting M2 polarization states of microglia have found to be beneficial in attenuating neuropsychiatric disorders (Table 18.1) as well as neurodegenerative diseases (Table 18.2). However, studies on regional heterogeneity of microglia pose a challenge for these therapies, and it directs extensive research toward understanding microglial function in region, sex, and disease-specific manner.

Acknowledgments The financial assistance provided by the Department of Biotechnology (DBT), Govt. of India, is acknowledged. The authors also acknowledge financial assistance provided under the Promotion of University Research and Scientific Excellence (PURSE) by Department of Science and Technology (DST) New Delhi and under University Grants Commission (UGC) under Special Assistance Program (SAP) of UGC (DRS Phase-II).

Conflict of Interest The authors state no conflict of interest.

References

Ahmed S, Kwatra M, Ranjan Panda S et al (2021) Andrographolide suppresses NLRP3 inflammasome activation in microglia through induction of parkin-mediated mitophagy in in-vitro and in-vivo models of Parkinson disease. Brain Behav Immun 91:142–158. https://doi.org/10.1016/j.bbi.2020.09.017

Apolloni S, Fabbrizio P, Parisi C et al (2016) Clemastine confers neuroprotection and induces an anti-inflammatory phenotype in SOD1G93A mouse model of amyotrophic lateral sclerosis. Mol Neurobiol 53:518–531. https://doi.org/10.1007/s12035-014-9019-8

Arioz BI, Tastan B, Tarakcioglu E et al (2019) Melatonin attenuates LPS-induced acute depressive-like behaviors and microglial NLRP3 inflammasome activation through the SIRT1/Nrf2 pathway. Front Immunol 10:1511. https://doi.org/10.3389/fimmu.2019.01511

Arnold T, Betsholtz C (2013) The importance of microglia in the development of the vasculature in the central nervous system. Vasc Cell 5:4

Aryanpour R, Pasbakhsh P, Zibara K et al (2017) Progesterone therapy induces an M1 to M2 switch in microglia phenotype and suppresses NLRP3 inflammasome in a cuprizone-induced demyelination mouse model. Int Immunopharmacol 51:131–139. https://doi.org/10.1016/j.intimp.2017.08.007

Babcock AA, Wirenfeldt M, Holm T et al (2006) Toll-like receptor 2 signaling in response to brain injury: an innate bridge to neuroinflammation. J Neurosci 26:12826–12837. https://doi.org/10.1523/JNEUROSCI.4937-05.2006

Balendra R, Isaacs AM (2018) C9orf72-mediated ALS and FTD: multiple pathways to disease. Nat Rev Neurol 14:544–558

Balestrino R, Schapira AHV (2020) Parkinson disease. Eur J Neurol 27:27–42

Barak V, Nisman B, Roisman I et al (1995) Changes in interleukin-1 β and soluble interleukin-2 receptor levels in csf and serum of schizophrenic patients. J Basic Clin Physiol Pharmacol 6:61–70. https://doi.org/10.1515/JBCPP.1995.6.1.61

Barati S, Ragerdi Kashani I, Moradi F et al (2019) Mesenchymal stem cell mediated effects on microglial phenotype in cuprizone-induced demyelination model. J Cell Biochem 120:13952–13964. https://doi.org/10.1002/jcb.28670

Barrientos RM, Frank MG, Hein AM et al (2009) Time course of hippocampal IL-1 β and memory consolidation impairments in aging rats following peripheral infection. Brain Behav Immun 23:46–54. https://doi.org/10.1016/j.bbi.2008.07.002

Bassett B, Subramaniyam S, Fan Y et al (2020) Minocycline alleviates depression-like symptoms by rescuing decrease in neurogenesis in dorsal hippocampus via blocking microglia activation/phagocytosis. Brain Behav Immun 91:519–530. https://doi.org/10.1016/j.bbi.2020.11.009

Benraiss A, Wang S, Herrlinger S et al (2016) Human glia can both induce and rescue aspects of disease phenotype in Huntington disease. Nat Commun 7:1–13. https://doi.org/10.1038/ncomms11758

Béraud D, Twomey M, Bloom B et al (2011) α-Synuclein alters toll-like receptor expression. Front Neurosci 5:80. https://doi.org/10.3389/fnins.2011.00080

Berkiks I, Benmhammed H, Mesfioui A et al (2018) Postnatal melatonin treatment protects against affective disorders induced by early-life immune stimulation by reducing the microglia cell activation and oxidative stress. Int J Neurosci 128:495–504. https://doi.org/10.1080/00207454.2017.1398156

Berkiks I, Garcia-Segura LM, Nassiri A et al (2019a) The sex differences of the behavior response to early life immune stimulation: microglia and astrocytes involvement. Physiol Behav 199:386–394. https://doi.org/10.1016/j.physbeh.2018.11.037

Berkiks I, Mesfioui A, Ouichou A et al (2019b) Affective behavior shows sex differences in mid-adulthood rats following postnatal immune stimulation. Neuroscience 421:69–81. https://doi.org/10.1016/j.neuroscience.2019.09.014

Bertram L, Tanzi RE (2009) Genome-wide association studies in Alzheimer's disease. Hum Mol Genet 18:R137. https://doi.org/10.1093/hmg/ddp406

Bjelobaba I, Savic D, Lavrnja I (2017) Multiple sclerosis and neuroinflammation: the overview of current and prospective therapies. Curr Pharm Des 23:693–730. https://doi.org/10.2174/1381612822666161214153108

Björkqvist M, Wild EJ, Thiele J et al (2008) A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. J Exp Med 205:1869–1877. https://doi.org/10.1084/jem.20080178

Bok E, Chung YC, Kim KS et al (2018) Modulation of M1/M2 polarization by capsaicin contributes to the survival of dopaminergic neurons in the lipopolysaccharide-lesioned substantia nigra in vivo. Exp Mol Med 50(7):1–14. https://doi.org/10.1038/s12276-018-0111-4

Calvello R, Cianciulli A, Nicolardi G et al (2017) Vitamin D treatment attenuates neuroinflammation and dopaminergic neurodegeneration in an animal model of Parkinson's disease, shifting M1 to M2 microglia responses. J Neuroimmune Pharmacol 12:327–339. https://doi.org/10.1007/s11481-016-9720-7

Chakraborty J, Singh R, Dutta D et al (2014) Quercetin improves behavioral deficiencies, restores astrocytes and microglia, and reduces serotonin metabolism in 3-nitropropionic acid-induced rat model of huntington's disease. CNS Neurosci Ther 20:10–19. https://doi.org/10.1111/cns.12189

Chang KH, Wu YR, Chen YC, Chen CM (2015) Plasma inflammatory biomarkers for Huntington's disease patients and mouse model. Brain Behav Immun 44:121–127. https://doi.org/10.1016/j.bbi.2014.09.011

Chaves Filho AJM, Cunha NL, Patrícia de Araújo Rodrigues, et al (2021) Doxycycline reverses cognitive impairment, neuroinflammation and oxidative imbalance induced by D-amphetamine mania model in mice: a promising drug repurposing for bipolar disorder treatment? Eur Neuropsychopharmacol 42:57–74. doi: https://doi.org/10.1016/j.euroneuro.2020.11.007

Chen T, Hou R, Xu S, Wu C (2015) Donepezil regulates 1-Methyl-4-phenylpyridinium-induced microglial polarization in Parkinson's disease. ACS Chem Neurosci 6:1708–1714. https://doi.org/10.1021/acschemneuro.5b00026

Cherry JD, Olschowka JA, O'Banion MK (2014) Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. J Neuroinflammation 11:98

Chien C-H, Lee M-J, Liou H-C et al (2016) Microglia-derived cytokines/chemokines are involved in the enhancement of LPS-induced loss of nigrostriatal dopaminergic neurons in DJ-1 knockout mice. PLoS One 11:e0151569. https://doi.org/10.1371/journal.pone.0151569

Cipollina G, Serej AD, Di Nolfi G et al (2020) Heterogeneity of neuroinflammatory responses in amyotrophic lateral sclerosis: a challenge or an opportunity? Int J Mol Sci 21:1–21

Claes C, Van Den Daele J, Boon R et al (2019) Human stem cell–derived monocytes and microglia-like cells reveal impaired amyloid plaque clearance upon heterozygous or homozygous loss of TREM2. Alzheimers Dement 15:453–464. https://doi.org/10.1016/j.jalz.2018.09.006

Colonna M, Butovsky O (2017) Microglia function in the central nervous system during health and neurodegeneration. Annu Rev Immunol 35:441–468

Crotti A, Glass CK (2015) The choreography of neuroinflammation in Huntington's disease. Trends Immunol 36:364–373

Cui W, Sun C, Ma Y et al (2020) Inhibition of TLR4 induces M2 microglial polarization and provides neuroprotection via the NLRP3 inflammasome in Alzheimer's disease. Front Neurosci 14:444. https://doi.org/10.3389/fnins.2020.00444

Dasgupta S, Jana M, Liu X, Pahan K (2002) Myelin basic protein-primed T cells induce nitric oxide synthase in microglial cells. Implications for multiple sclerosis. J Biol Chem 277:39,327–39,333. https://doi.org/10.1074/jbc.M111841200

De Picker LJ, Morrens M, Chance SA, Boche D (2017) Microglia and brain plasticity in acute psychosis and schizophrenia illness course: a meta-review. Front Psychiatry 8:238. https://doi.org/10.3389/fpsyt.2017.00238

Debye B, Schmülling L, Zhou L et al (2018) Neurodegeneration and NLRP3 inflammasome expression in the anterior thalamus of SOD1(G93A) ALS mice. Brain Pathol 28:14–27. https://doi.org/10.1111/bpa.12467

DeMaagd G, Philip A (2015) Parkinson's disease and its management part 1: disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis. P T 40:504–532

Deora V, Lee JD, Albornoz EA et al (2020) The microglial NLRP3 inflammasome is activated by amyotrophic lateral sclerosis proteins. Glia 68:407–421. https://doi.org/10.1002/glia.23728

Dong Y, Li X, Cheng J, Hou L (2019) Drug development for Alzheimer's disease: microglia induced neuroinflammation as a target? Int J Mol Sci 20(3):558. https://doi.org/10.3390/ijms20030558

Du Y, Luo M, Du Y et al (2020) Liquiritigenin decreases Aβ levels and ameliorates cognitive decline by regulating microglia M1/M2 transformation in AD mice. Neurotox Res 39(2):349–358. https://doi.org/10.1007/s12640-020-00284-z

Ebner F, Brandt C, Thiele P et al (2013) Microglial activation milieu controls regulatory T cell responses. J Immunol 191:5594–5602. https://doi.org/10.4049/jimmunol.1203331

El Hajj H, Savage JC, Bisht K et al (2019) Ultrastructural evidence of microglial heterogeneity in Alzheimer's disease amyloid pathology. J Neuroinflammation 16(1):87. https://doi.org/10.1186/s12974-019-1473-9

Esshili A, Manitz MP, Freund N, Juckel G (2020) Induction of inducible nitric oxide synthase expression in activated microglia and astrocytes following pre- and postnatal immune challenge in an animal model of schizophrenia. Eur Neuropsychopharmacol 35:100–110. https://doi.org/10.1016/j.euroneuro.2020.04.002

Ferreira SA, Romero-Ramos M (2018) Microglia response during Parkinson's disease: alpha-synuclein intervention. Front Cell Neurosci 12:247. https://doi.org/10.3389/fncel.2018.00247

Garay PA, Hsiao EY, Patterson PH, McAllister AK (2013) Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. Brain Behav Immun 31:54–68. https://doi.org/10.1016/j.bbi.2012.07.008

Geloso MC, Corvino V, Marchese E et al (2017) The dual role of microglia in ALS: mechanisms and therapeutic approaches. Front Aging Neurosci 9:242. https://doi.org/10.3389/fnagi.2017.00242

Giorgini F, Möller T, Kwan W et al (2008) Histone deacetylase inhibition modulates kynurenine pathway activation in yeast, microglia, and mice expressing a mutant huntingtin fragment. J Biol Chem 283:7390–7400. https://doi.org/10.1074/jbc.M708192200

Gong W, Zhang S, Zong Y et al (2019) Involvement of the microglial NLRP3 inflammasome in the anti-inflammatory effect of the antidepressant clomipramine. J Affect Disord 254:15–25. https://doi.org/10.1016/j.jad.2019.05.009

Gordon R, Albornoz EA, Christie DC et al (2018) Inflammasome inhibition prevents -synuclein pathology and dopaminergic neurodegeneration in mice. Sci Transl Med 1010(465):eaah4066. https://doi.org/10.1126/scitranslmed.aah4066

Grabert K, Michoel T, Karavolos MH et al (2016) Microglial brain regionâ 'dependent diversity and selective regional sensitivities to aging. Nat Neurosci 19:504–516. https://doi.org/10.1038/nn.4222

Grottelli S, Mezzasoma L, Scarpelli P et al (2019) Cyclo(his-pro) inhibits NLRP3 inflammasome cascade in ALS microglial cells. Mol Cell Neurosci 94:23–31. https://doi.org/10.1016/j.mcn.2018.11.002

Gudi V, Moharregh-Khiabani D, Skripuletz T et al (2009) Regional differences between grey and white matter in cuprizone induced demyelination. Brain Res 1283:127–138. https://doi.org/10.1016/j.brainres.2009.06.005

Guerrero BL, Sicotte NL (2020) Microglia in multiple sclerosis: friend or foe? Front Immunol 11:374. https://doi.org/10.3389/fimmu.2020.00374

Gugliandolo A, Giacoppo S, Bramanti P, Mazzon E (2018) NLRP3 inflammasome activation in a transgenic amyotrophic lateral sclerosis model. Inflammation 41:93–103. https://doi.org/10.1007/s10753-017-0667-5

Guo M, Wang J, Zhao Y et al (2020a) Microglial exosomes facilitate a-synuclein transmission in Parkinson's disease. Brain 143:1476–1497. https://doi.org/10.1093/brain/awaa090

Guo Y, Gan X, Zhou H et al (2020b) Fingolimod suppressed the chronic unpredictable mild stress-induced depressive-like behaviors via affecting microglial and NLRP3 inflammasome activation. Life Sci 263:118,582. https://doi.org/10.1016/j.lfs.2020.118582
Hamza TH, Zabetian CP, Tenesa A et al (2010) Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. Nat Genet 42:781. https://doi.org/10.1038/ng.642
Han J, Wang M, Ren M, Lou H (2017) Contributions of triggering-receptor-expressed-on-myeloid-cells-2 to neurological diseases. Int J Neurosci 127:368–375
Han X, Sun S, Sun Y et al (2019) Small molecule-driven NLRP3 inflammation inhibition via interplay between ubiquitination and autophagy: implications for Parkinson disease. Autophagy 15:1860–1881. https://doi.org/10.1080/15548627.2019.1596481
Hansen DV, Hanson JE, Sheng M (2018) Microglia in Alzheimer's disease. J Cell Biol 217:459–472
Hartwig FP, Borges MC, Horta BL et al (2017) Inflammatory biomarkers and risk of schizophrenia: a 2-sample mendelian randomization study. JAMA Psychiatry 74:1226–1233. https://doi.org/10.1001/jamapsychiatry.2017.3191
Hashemiaghdam A, Mroczek M (2020) Microglia heterogeneity and neurodegeneration: the emerging paradigm of the role of immunity in Alzheimer's disease. J Neuroimmunol 341:577185. https://doi.org/10.1016/j.jneuroim.2020.577185
Heneka MT, Kummer MP, Stutz A et al (2013) NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493:674–678. https://doi.org/10.1038/nature11729
Hickman S, Izzy S, Sen P et al (2018) Microglia in neurodegeneration. Nat Neurosci 21:1359–1369
Hill SL, Shao L, Beasley CL (2020) Diminished levels of the chemokine fractalkine in post-mortem prefrontal cortex in schizophrenia but not bipolar disorder. World J Biol Psychiatry 22(2):94–103. https://doi.org/10.1080/15622975.2020.1755451
Ho MS (2019) Microglia in parkinson's disease. In: Advances in experimental medicine and biology. Springer New York LLC, pp 335–353
Holtman IR, Skola D, Glass CK (2017) Transcriptional control of microglia phenotypes in health and disease. J Clin Invest 127:3220–3229
Huang C, Wang P, Xu X et al (2018) The ketone body metabolite β-hydroxybutyrate induces an antidepression-associated ramification of microglia via HDACs inhibition-triggered Akt-small RhoGTPase activation. Glia 66:256–278. https://doi.org/10.1002/glia.23241
Ilieva H, Polymenidou M, Cleveland DW (2009) Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. J Cell Biol 187:761–772
Isgren A, Sellgren C, Ekman CJ et al (2017) Markers of neuroinflammation and neuronal injury in bipolar disorder: relation to prospective clinical outcomes. Brain Behav Immun 65:195–201. https://doi.org/10.1016/j.bbi.2017.05.002
Ising C, Venegas C, Zhang S et al (2019) NLRP3 inflammasome activation drives tau pathology. Nature 575:669–673. https://doi.org/10.1038/s41586-019-1769-z
Jakobsson J, Bjerke M, Sahebi S et al (2015) Monocyte and microglial activation in patients with mood-stabilized bipolar disorder. J Psychiatry Neurosci 40:250–258. https://doi.org/10.1503/jpn.140183
Jansen AHP, van Hal M, op den Kelder IC et al (2017) Frequency of nuclear mutant huntingtin inclusion formation in neurons and glia is cell-type-specific. Glia 65:50–61. https://doi.org/10.1002/glia.23050
Jeon SW, Kim YK (2018) The role of neuroinflammation and neurovascular dysfunction in major depressive disorder. J Inflamm Res 11:179–192
Jesulola E, Micalos P, Baguley IJ (2018) Understanding the pathophysiology of depression: from monoamines to the neurogenesis hypothesis model—are we there yet? Behav Brain Res 341:79–90
Jiang DQ, Ma YJ, Wang Y et al (2019) Microglia activation induces oxidative injury and decreases SIRT3 expression in dopaminergic neuronal cells. J Neural Transm 126:559–568. https://doi.org/10.1007/s00702-019-02005-z

Jin X, Liu MY, Zhang DF et al (2019) Natural products as a potential modulator of microglial polarization in neurodegenerative diseases. Pharmacol Res 145:104253. https://doi.org/10.1016/j.phrs.2019.104253

Kamimura D, Tanaka Y, Hasebe R, Murakami M (2020) Bidirectional communication between neural and immune systems. Int Immunol 32:693–701. https://doi.org/10.1093/intimm/dxz083

Karlsson H, Dalman C (2020) Epidemiological studies of prenatal and childhood infection and schizophrenia. Curr Top Behav Neurosci 44:35–47

Karperien A, Ahammer H, Jelinek HF (2013) Quantitating the subtleties of microglial morphology with fractal analysis. Front Cell Neurosci 7:1–34

Kelley N, Jeltema D, Duan Y, He Y (2019) The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. Int J Mol Sci 20(13):3328. https://doi.org/10.3390/ijms20133328

Kim WG, Mohney RP, Wilson B et al (2000) Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. J Neurosci 20:6309–6316. https://doi.org/10.1523/jneurosci.20-16-06309.2000

Kim HK, Chen W, Andreazza AC (2015) The potential role of the NLRP3 inflammasome as a link between mitochondrial complex I dysfunction and inflammation in bipolar disorder. Neural Plast 2015:408136. https://doi.org/10.1155/2015/408136

Kim CC, Nakamura MC, Hsieh CL (2016a) Brain trauma elicits non-canonical macrophage activation states. J Neuroinflammation 13(1):117. https://doi.org/10.1186/s12974-016-0581-z

Kim HK, Andreazza AC, Elmi N et al (2016b) Nod-like receptor pyrin containing 3 (NLRP3) in the post-mortem frontal cortex from patients with bipolar disorder: a potential mediator between mitochondria and immune-activation. J Psychiatr Res 72:43–50. https://doi.org/10.1016/j.jpsychires.2015.10.015

Kim YE, Lai TT, Kim YJ, Jeon B (2020) Preferential microglial activation associated with pathological alpha synuclein transmission. J Clin Neurosci 81:469–476. https://doi.org/10.1016/j.jocn.2020.09.027

Kraft AD, Kaltenbach LS, Lo DC, Harry GJ (2012) Activated microglia proliferate at neurites of mutant huntingtin-expressing neurons. Neurobiol Aging 33:621.e17–621.e33. https://doi.org/10.1016/j.neurobiolaging.2011.02.015

Kumar M, Sandhir R (2019) Hydrogen sulfide suppresses homocysteine-induced glial activation and inflammatory response. Nitric Oxide—Biol Chem 90:15–28. https://doi.org/10.1016/j.niox.2019.05.008

Kumar M, Arora P, Sandhir R (2020) Hydrogen sulfide reverses LPS-induced behavioral deficits by suppressing microglial activation and promoting M2 polarization. J Neuroimmune Pharmacol 16(2):483–499. https://doi.org/10.1007/s11481-020-09920-z

Lanz TA, Reinhart V, Sheehan MJ et al (2019) Postmortem transcriptional profiling reveals widespread increase in inflammation in schizophrenia: a comparison of prefrontal cortex, striatum, and hippocampus among matched tetrads of controls with subjects diagnosed with schizophrenia, bipolar or major depressive disorder. Transl Psychiatry 9(1):151. https://doi.org/10.1038/s41398-019-0492-8

Lavisse S, Goutal S, Wimberley C et al (2021) Increased microglial activation in patients with Parkinson disease using [18F]-DPA714 TSPO PET imaging. Parkinsonism Relat Disord 82:29–36. https://doi.org/10.1016/j.parkreldis.2020.11.011

Lee J, Hyeon SJ, Im H et al (2016) Astrocytes and microglia as non-cell autonomous players in the pathogenesis of ALS. Exp Neurobiol 25:233–240

Lei Y, Chen CJ, Yan XX et al (2017) Early-life lipopolysaccharide exposure potentiates forebrain expression of NLRP3 inflammasome proteins and anxiety-like behavior in adolescent rats. Brain Res 1671:43–54. https://doi.org/10.1016/j.brainres.2017.06.014

Lenz KM, Nelson LH (2018) Microglia and beyond: innate immune cells as regulators of brain development and behavioral function. Front Immunol 9:698. https://doi.org/10.3389/fimmu.2018.00698

Lewis CA, Manning J, Rossi F, Krieger C (2012) The neuroinflammatory response in ALS: the roles of microglia and T cells. Neurol Res Int 2012:803701. https://doi.org/10.1155/2012/803701

Li R, Wang X, Qin T et al (2016) Apigenin ameliorates chronic mild stress-induced depressive behavior by inhibiting interleukin-1β production and NLRP3 inflammasome activation in the rat brain. Behav Brain Res 296:318–325. https://doi.org/10.1016/j.bbr.2015.09.031

Li Z, Liu F, He X et al (2019) Exosomes derived from mesenchymal stem cells attenuate inflammation and demyelination of the central nervous system in EAE rats by regulating the polarization of microglia. Int Immunopharmacol 67:268–280. https://doi.org/10.1016/j.intimp.2018.12.001

Lively S, Schlichter LC (2018) Microglia responses to pro-inflammatory stimuli (LPS, IFNγ +TNFα) and reprogramming by resolving cytokines (IL-4, IL-10). Front Cell Neurosci 12:215. https://doi.org/10.3389/fncel.2018.00215

Loram LC, Sholar PW, Taylor FR et al (2012) Sex and estradiol influence glial pro-inflammatory responses to lipopolysaccharide in rats. Psychoneuroendocrinology 37:1688–1699. https://doi.org/10.1016/j.psyneuen.2012.02.018

Luo C, Jian C, Liao Y et al (2017) The role of microglia in multiple sclerosis. Neuropsychiatr Dis Treat 13:1661–1667

Lurie DI (2018) An integrative approach to neuroinflammation in psychiatric disorders and neuropathic pain. J Exp Neurosci 12:1–11. https://doi.org/10.1177/1179069518793639

Lyu J, Jiang X, Leak RK et al (2020) Microglial responses to brain injury and disease: functional diversity and new opportunities. Transl Stroke Res 12:474–495

Machado-Pereira M, Santos T, Ferreira L et al (2017) Anti-inflammatory strategy for M2 microglial polarization using retinoic acid-loaded nanoparticles. Mediators Inflamm 2017:6742427. https://doi.org/10.1155/2017/6742427

Madore C, Joffre C, Delpech JC et al (2013) Early morphofunctional plasticity of microglia in response to acute lipopolysaccharide. Brain Behav Immun 34:151–158. https://doi.org/10.1016/j.bbi.2013.08.008

Malhotra S, Costa C, Eixarch H et al (2020) NLRP3 inflammasome as prognostic factor and therapeutic target in primary progressive multiple sclerosis patients. Brain 143:1414–1430. https://doi.org/10.1093/brain/awaa084

Maniatis S, Äijö T, Vickovic S et al (2019) Spatiotemporal dynamics of molecular pathology in amyotrophic lateral sclerosis. Science (80-) 364:89–93. https://doi.org/10.1126/science.aav9776

Marinelli S, Basilico B, Marrone MC, Ragozzino D (2019) Microglia-neuron crosstalk: signaling mechanism and control of synaptic transmission. Semin Cell Dev Biol 94:138–151

Marques TR, Ashok AH, Pillinger T et al (2019) Neuroinflammation in schizophrenia: meta-analysis of in vivo microglial imaging studies. Psychol Med 49:2186–2196. https://doi.org/10.1017/S0033291718003057

Masuda T, Sankowski R, Staszewski O, Prinz M (2020) Microglia heterogeneity in the single-cell era. Cell Rep 30:1271–1281

McKenzie BA, Mamik MK, Saito LB et al (2018) Caspase-1 inhibition prevents glial inflammasome activation and pyroptosis in models of multiple sclerosis. Proc Natl Acad Sci U S A 115:E6065–E6074. https://doi.org/10.1073/pnas.1722041115

Mehra S, Sahay S, Maji SK (2019) α-Synuclein misfolding and aggregation: implications in Parkinson's disease pathogenesis. Biochim. Biophys Acta Proteins Proteomics 1867:890–908

Miao EA, Rajan JV, Aderem A (2011) Caspase-1-induced pyroptotic cell death. Immunol Rev 243:206–214

Miao J, Wang F, Wang R et al (2019) Pleiotrophin regulates functional heterogeneity of microglia cells in EAE animal models of multiple sclerosis by activating CCr-7/CD206 molecules and functional cytokines—PubMed. Am J Transl Res 15:2013–2027

Moehle MS, Webber PJ, Tse T et al (2012) LRRK2 inhibition attenuates microglial inflammatory responses. J Neurosci 32:1602–1611. https://doi.org/10.1523/JNEUROSCI.5601-11.2012

Monji A, Kato T, Kanba S (2009) Cytokines and schizophrenia: microglia hypothesis of schizophrenia. Psychiatry Clin Neurosci 63:257–265. https://doi.org/10.1111/j.1440-1819.2009.01945.x

Na KS, Jung HY, Kim YK (2014) The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry 48:277–286

Nagatsu T, Mogi M, Ichinose H, Togari A (2000) Changes in cytokines and neurotrophins in Parkinson's disease. J Neural Transmission, Supplement. Springer Wien, pp. 277–290

Nishiguchi T, Iwata M, Kajitani N et al (2021) Stress increases blood beta-hydroxybutyrate levels and prefrontal cortex NLRP3 activity jointly in a rodent model. Neuropsychopharmacol Rep 41 (2):159–167. https://doi.org/10.1002/npr2.12164

O'Loughlin E, Pakan JMP, Yilmazer-Hanke D, McDermott KW (2017) Acute in utero exposure to lipopolysaccharide induces inflammation in the pre- and postnatal brain and alters the glial cytoarchitecture in the developing amygdala. J Neuroinflammation 14(1):212. https://doi.org/10.1186/s12974-017-0981-8

O'Loughlin E, Madore C, Lassmann H, Butovsky O (2018) Microglial phenotypes and functions in multiple sclerosis. Cold Spring Harb Perspect Med 8(2):a028993. https://doi.org/10.1101/cshperspect.a028993

O'Rourke JG, Bogdanik L, Yáñez A et al (2016) C9orf72 is required for proper macrophage and microglial function in mice. Science 351(6279):1324–1329. https://doi.org/10.1126/science.aaf1064

Ohgidani M, Kato TA, Haraguchi Y et al (2017) Microglial CD206 gene has potential as a state marker of bipolar disorder. Front Immunol 7:676. https://doi.org/10.3389/fimmu.2016.00676

Ohgomori T, Yamada J, Takeuchi H et al (2016) Comparative morphometric analysis of microglia in the spinal cord of SOD1G93A transgenic mouse model of amyotrophic lateral sclerosis. Eur J Neurosci 43:1340–1351. https://doi.org/10.1111/ejn.13227

Orlovska-Waast S, Köhler-Forsberg O, Brix SW et al (2019) Cerebrospinal fluid markers of inflammation and infections in schizophrenia and affective disorders: a systematic review and meta-analysis. Mol Psychiatry 24:869–887. https://doi.org/10.1038/s41380-018-0220-4

Paldino E, D'Angelo V, Laurenti D et al (2020a) Modulation of inflammasome and Pyroptosis by Olaparib, a PARP-1 inhibitor, in the R6/2 mouse model of Huntington's disease. Cell 9 (10):2286. https://doi.org/10.3390/cells9102286

Paldino E, D'Angelo V, Sancesario G, Fusco FR (2020b) Pyroptotic cell death in the R6/2 mouse model of Huntington's disease: new insight on the inflammasome. Cell Death Dis 6:69. https://doi.org/10.1038/s41420-020-00293-z

Pandey GN, Rizavi HS, Bhaumik R, Ren X (2019) Innate immunity in the postmortem brain of depressed and suicide subjects: role of toll-like receptors. Brain Behav Immun 75:101–111. https://doi.org/10.1016/j.bbi.2018.09.024

Paolicelli RC, Bolasco G, Pagani F et al (2011) Synaptic pruning by microglia is necessary for normal brain development. Science (80-) 333:1456–1458. https://doi.org/10.1126/science.1202529

Park GH, Noh H, Shao Z et al (2020) Activated microglia cause metabolic disruptions in developmental cortical interneurons that persist in interneurons from individuals with schizophrenia. Nat Neurosci 23:1352–1364. https://doi.org/10.1038/s41593-020-00724-1

Patro I, Nagayach A, Sinha S, Patro N (2016) General physiology and pathophysiology of microglia during neuroinflammation. Inflamm Common Link Brain Pathol:17–42. https://doi.org/10.1007/978-981-10-1711-7_2

Pavese N, Gerhard A, Tai YF et al (2006) Microglial activation correlates with severity in Huntington disease: a clinical and PET study. Neurology 66:1638–1643. https://doi.org/10.1212/01.wnl.0000222734.56412.17

Pereira CF, Santos AE, Moreira PI et al (2019) Is Alzheimer's disease an inflammasomopathy? Ageing Res Rev 56:100,966

Perry VH, Holmes C (2014) Microglial priming in neurodegenerative disease. Nat Rev Neurol 10: 217–224

Ponath G, Park C, Pitt D (2018) The role of astrocytes in multiple sclerosis. Front Immunol 9:1
Ramanan VK, Risacher SL, Nho K et al (2015) GWAS of longitudinal amyloid accumulation on 18F-florbetapir PET in Alzheimer's disease implicates microglial activation gene IL1RAP. Brain 138:3076–3088. https://doi.org/10.1093/brain/awv231
Rodrigues FB, Byrne LM, McColgan P et al (2016) Cerebrospinal fluid inflammatory biomarkers reflect clinical severity in Huntington's disease. PLoS One 11(9):e0163479. https://doi.org/10.1371/journal.pone.0163479
Rossetti AC, Paladini MS, Trepci A et al (2019) Differential neuroinflammatory response in male and female mice: a role for BDNF. Front Mol Neurosci 12:166. https://doi.org/10.3389/fnmol.2019.00166
Rossi S, Cozzolino M, Carrì MT (2016) Old versus new mechanisms in the pathogenesis of ALS. In: Brain pathology. Blackwell Publishing Ltd, pp 276–286
Roy A, Liu X, Pahan K (2007) Myelin basic protein-primed T cells induce neurotrophins in glial cells via α5β3 integrin. J Biol Chem 282:32222–32232. https://doi.org/10.1074/jbc.M702899200
Ryu JK, Kim SU, McLarnon JG (2004) Blockade of quinolinic acid-induced neurotoxicity by pyruvate is associated with inhibition of glial activation in a model of Huntington's disease. Exp Neurol 187:150–159. https://doi.org/10.1016/j.expneurol.2004.01.006
Sakrajda K, Szczepankiewicz A (2021) Inflammation-related changes in mood disorders and the immunomodulatory role of lithium. Int J Mol Sci 22:1–15
Sansom DM (2000) CD28, CTLA-4 and their ligands: who does what and to whom? Immunology 101:169–177
Sapp E, Kegel KB, Aronin N et al (2001) Early and progressive accumulation of reactive microglia in the Huntington disease brain. J Neuropathol Exp Neurol 60:161–172. https://doi.org/10.1093/jnen/60.2.161
Sarkar S, Rokad D, Malovic E et al (2019) Manganese activates NLRP3 inflammasome signaling and propagates exosomal release of ASC in microglial cells. Sci Signal 12(563):eaat9900. https://doi.org/10.1126/scisignal.aat9900
Sato K (2015) Effects of microglia on neurogenesis. Glia 63:1394–1405
Sawa A, Sedlak TW (2016) Oxidative stress and inflammation in schizophrenia. Schizophr Res 176:1–2
Scaini G, Barichello T, Fries GR et al (2019) TSPO upregulation in bipolar disorder and concomitant downregulation of mitophagic proteins and NLRP3 inflammasome activation. Neuropsychopharmacology 44:1291–1299. https://doi.org/10.1038/s41386-018-0293-4
Schirmer L, Velmeshev D, Holmqvist S et al (2019) Neuronal vulnerability and multilineage diversity in multiple sclerosis. Nature 573:75–82. https://doi.org/10.1038/s41586-019-1404-z
Sellgren CM, Gracias J, Watmuff B et al (2019) Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. Nat Neurosci 22:374–385. https://doi.org/10.1038/s41593-018-0334-7
Seney ML, Huo Z, Cahill K et al (2018) Opposite molecular signatures of depression in men and women. Biol Psychiatry 84:18–27. https://doi.org/10.1016/j.biopsych.2018.01.017
Sheeler C, Rosa JG, Ferro A et al (2020) Glia in neurodegeneration: the housekeeper, the defender and the perpetrator. Int J Mol Sci 21:1–16
Silverman HA, Dancho M, Regnier-Golanov A et al (2014) Brain region-specific alterations in the gene expression of cytokines, immune cell markers and cholinergic system components during peripheral endotoxin-induced inflammation. Mol Med 20:601–611. https://doi.org/10.2119/molmed.2014.00147
Ślusarczyk J, Trojan E, Głombik K et al (2018) Targeting the NLRP3 inflammasome-related pathways via tianeptine treatment-suppressed microglia polarization to the M1 phenotype in lipopolysaccharide-stimulated cultures. Int J Mol Sci 19(7):1965. https://doi.org/10.3390/ijms19071965

Sneeboer MAM, Snijders GJLJ, Berdowski WM et al (2019) Microglia in post-mortem brain tissue of patients with bipolar disorder are not immune activated. Transl Psychiatry 9(1):153. https://doi.org/10.1038/s41398-019-0490-x

Song L, Pei L, Yao S et al (2017) NLRP3 inflammasome in neurological diseases, from functions to therapies. Front Cell Neurosci 11:63. https://doi.org/10.3389/fncel.2017.00063

Song Y, Sun R, Ji Z et al (2018) Perilla aldehyde attenuates CUMS-induced depressive-like behaviors via regulating TXNIP/TRX/NLRP3 pathway in rats. Life Sci 206:117–124. https://doi.org/10.1016/j.lfs.2018.05.038

Stelzmann RA, Norman Schnitzlein H, Reed Murtagh F (1995) An English translation of Alzheimer's 1907 paper, "über eine eigenartige erkankung der hirnrinde". Clin Anat 8:429–431. https://doi.org/10.1002/ca.980080612

Su F, Yi H, Xu L, Zhang Z (2015) Fluoxetine and S-citalopram inhibit M1 activation and promote M2 activation of microglia in vitro. Neuroscience 294:60–68. https://doi.org/10.1016/j.neuroscience.2015.02.028

Suárez-Calvet M, Caballero MÁA, Kleinberger G et al (2016) Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. Sci Transl Med 8(369):369ra178. https://doi.org/10.1126/scitranslmed.aag1767

Suzuki H, Ohgidani M, Kuwano N et al (2019) Suicide and microglia: recent findings and future perspectives based on human studies. Front Cell Neurosci 13:31. https://doi.org/10.3389/fncel.2019.00031

Tai YF, Pavese N, Gerhard A et al (2007) Microglial activation in presymptomatic Huntington's disease gene carriers. Brain 130:1759–1766. https://doi.org/10.1093/brain/awm044

Tan YL, Yuan Y, Tian L (2020) Microglial regional heterogeneity and its role in the brain. Mol Psychiatry 25:351–367

Tang Y (2018) Editorial: microglial polarization in the pathogenesis and therapeutics of neurodegenerative diseases. Front Aging Neurosci 10:154. https://doi.org/10.3389/fnagi.2018.00154

Tang Y, Le W (2016) Differential roles of M1 and M2 microglia in neurodegenerative diseases. Mol Neurobiol 53:1181–1194. https://doi.org/10.1007/s12035-014-9070-5

Tian L, Ma L, Kaarela T, Li Z (2012) Neuroimmune crosstalk in the central nervous system and its significance for neurological diseases. J Neuroinflammation 9:155. https://doi.org/10.1186/1742-2094-9-155

Tomasik J, Rahmoune H, Guest PC, Bahn S (2016) Neuroimmune biomarkers in schizophrenia. Schizophr Res 176:3–13

Tremblay MÈ, Majewska AK (2011) A role for microglia in synaptic plasticity? Commun Integr Biol 4:220–222. https://doi.org/10.4161/cib.4.2.14506

Troubat R, Barone P, Leman S et al (2021) Neuroinflammation and depression: a review. Eur J Neurosci 53:151–171. https://doi.org/10.1111/ejn.14720

Valekova I, Jarkovska K, Kotrcova E et al (2016) Revelation of the IFNα, IL-10, IL-8 and IL-1β as promising biomarkers reflecting immuno-pathological mechanisms in porcine Huntington's disease model. J Neuroimmunol 293:71–81. https://doi.org/10.1016/j.jneuroim.2016.02.012

Van Eeden WA, van Hemert AM, Carlier IVE et al (2020) Basal and LPS-stimulated inflammatory markers and the course of individual symptoms of depression. Transl Psychiatry 10:1–12. https://doi.org/10.1038/s41398-020-00920-4

Voet S, Prinz M, van Loo G (2019) Microglia in central nervous system inflammation and multiple sclerosis pathology. Trends Mol Med 25:112–123

Walker FO (2007) Huntington's disease. Lancet 369:218–228

Wijesekera LC, Leigh PN (2009) Amyotrophic lateral sclerosis. Orphanet J Rare Dis 4:3. https://doi.org/10.1186/1750-1172-4-3

Woelfer M, Kasties V, Kahlfuss S, Walter M (2019) The role of depressive subtypes within the neuroinflammation hypothesis of major depressive disorder. Neuroscience 403:93–110

Yamanashi T, Iwata M, Kamiya N et al (2017) Beta-hydroxybutyrate, an endogenic NLRP3 inflammasome inhibitor, attenuates stress-induced behavioral and inflammatory responses. Sci Rep 7(1):7677. https://doi.org/10.1038/s41598-017-08055-1

Yan A, Liu Z, Song L et al (2019) Idebenone alleviates neuroinflammation and modulates microglial polarization in LPS-stimulated BV2 cells and MPTP-induced Parkinson's disease mice. Front Cell Neurosci 12:529. https://doi.org/10.3389/fncel.2018.00529

Yang HM, Yang S, Huang SS et al (2017) Microglial activation in the pathogenesis of Huntington's disease. Front Aging Neurosci 9:193. https://doi.org/10.3389/fnagi.2017.00193

Yang Z, Kuboyama T, Tohda C (2019) Naringenin promotes microglial M2 polarization and Aβ degradation enzyme expression. Phyther Res 33:1114–1121. https://doi.org/10.1002/ptr.6305

Yanguas-Casás N (2020) Physiological sex differences in microglia and their relevance in neurological disorders. Neuroimmunol Neuroinflammation 7:13–22. https://doi.org/10.20517/2347-8659.2019.31

Yu-Taeger L, Stricker-Shaver J, Arnold K et al (2019) Intranasal administration of mesenchymal stem cells ameliorates the abnormal dopamine transmission system and inflammatory reaction in the R6/2 mouse model of Huntington disease. Cell 8:595. https://doi.org/10.3390/cells8060595

Zhang Y, He ML (2017) Deferoxamine enhances alternative activation of microglia and inhibits amyloid beta deposits in APP/PS1 mice. Brain Res 1677:86–92. https://doi.org/10.1016/j.brainres.2017.09.019

Zhang L, Previn R, Lu L et al (2018a) Crocin, a natural product attenuates lipopolysaccharide-induced anxiety and depressive-like behaviors through suppressing NF-kB and NLRP3 signaling pathway. Brain Res Bull 142:352–359. https://doi.org/10.1016/j.brainresbull.2018.08.021

Zhang L, Zhang J, You Z (2018b) Switching of the microglial activation phenotype is a possible treatment for depression disorder. Front Cell Neurosci 12:306. https://doi.org/10.3389/fncel.2018.00306

Zhang Y, Feng S, Nie K et al (2018c) TREM2 modulates microglia phenotypes in the neuroinflammation of Parkinson's disease. Biochem Biophys Res Commun 499:797–802. https://doi.org/10.1016/j.bbrc.2018.03.226

Zhang C, Zhang YP, Li YY et al (2019a) Minocycline ameliorates depressive behaviors and neuroimmune dysfunction induced by chronic unpredictable mild stress in the rat. Behav Brain Res 356:348–357. https://doi.org/10.1016/j.bbr.2018.07.001

Zhang L, Zheng H, Wu R et al (2019b) The effect of minocycline on amelioration of cognitive deficits and pro-inflammatory cytokines levels in patients with schizophrenia. Schizophr Res 212:92–98. https://doi.org/10.1016/j.schres.2019.08.005

Zhang L, Lu X, Gong L et al (2020a) Tetramethylpyrazine protects blood-spinal cord barrier integrity by modulating microglia polarization through activation of STAT3/SOCS3 and inhibition of NF-κB signaling pathways in experimental autoimmune encephalomyelitis mice. Cell Mol Neurobiol 41(4):417–431. https://doi.org/10.1007/s10571-020-00878-3

Zhang Y, Zhao Y, Zhang J, Yang G (2020b) Mechanisms of NLRP3 inflammasome activation: its role in the treatment of Alzheimer's disease. Neurochem Res 45:2560–2572

Zhao W, Beers DR, Henkel JS et al (2010) Extracellular mutant SOD1 induces microglial-mediated motoneuron injury. Glia 58:231–243. https://doi.org/10.1002/glia.20919

Zhao W, Beers DR, Bell S et al (2015) TDP-43 activates microglia through NF-κB and NLRP3 inflammasome. Exp Neurol 273:24–35. https://doi.org/10.1016/j.expneurol.2015.07.019

Zheng ZH, Tu JL, Li XH et al (2021) Neuroinflammation induces anxiety- and depressive-like behavior by modulating neuronal plasticity in the basolateral amygdala. Brain Behav Immun 91:505–518. https://doi.org/10.1016/j.bbi.2020.11.007

Zou YH, Guan PP, Zhang SQ et al (2020) Rofecoxib attenuates the pathogenesis of amyotrophic lateral sclerosis by alleviating cyclooxygenase-2-mediated mechanisms. Front Neurosci 14:817. https://doi.org/10.3389/fnins.2020.00817

Multidimensional Roles of Microglial Cells in Neuroviral Infections

Meghana Rastogi, Neha Pandey, Astha Shukla, Saurabh Singh, and Sunit K. Singh

Abstract

Microglial cells are the brain resident macrophages which are involved in maintaining CNS homeostasis. During CNS infections, the microglial cells get activated and trigger immune response. Neuroviral infections often lead to encephalitis, encephalopathy, and meningitis. This chapter highlights the roles of microglial cells during several neuroviral infections. Microglia, as the first responders to neuroviral infections, generate pro-inflammatory and antiviral response, affect the adaptive immune response and perturb the cell death pathways. The mechanisms behind most of these responses are poorly understood and require further studies in order to understand the pathophysiological mechanisms during neuroviral infections.

Keywords

Microglia · Neurotropic viruses · Neuroinflammation · Neuroviral infection

1 Introduction

Microglial cells are specialized cells in the CNS parenchyma which originate from myeloid precursor cells during the developmental process (Ginhoux et al. 2010). Microglial cells are extremely important during brain development and

M. Rastogi · N. Pandey · A. Shukla · S. K. Singh (✉)
Molecular Biology Unit, Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

S. Singh
Department of Orthopedic Surgery, Trauma Centre, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_19

Fig. 19.1 Physiological roles of microglial cells in the CNS: The microglial cells are sentinels of the CNS involved in immune surveillance, maintaining blood-brain barrier integrity, and clearance of apoptotic cells, thereby maintaining brain homeostasis. Microglial cells interact with neurons to perpetuate the myelination process, synaptic pruning, and synaptic stripping to sustain neural circuits. Dysregulation in the functioning of microglial cells results in neuronal loss and neuroinflammation

establishment of neural circuits (Bilimoria and Stevens 2015; Derecki et al. 2014). These cells are CNS resident macrophages involved in immune surveillance, phagocytosis of infected cells, and initiation of antiviral and pro-inflammatory responses. The microglial cells constantly surveillance brain, sense the disturbances and maintain the brain homeostasis. The resting microglial cells present a ramified morphology with constantly extending and retracting processes to monitor their surroundings (Nimmerjahn et al. 2005; Colonna and Butovsky 2017) (Fig. 19.1).

The neurotropic viruses have a propensity toward neural tissues, most commonly neurons. Neurotropic viruses enter into the CNS by either the "Trojan horse mechanism," axonal-retrograde transport, breaching the blood-brain barrier (BBB), through the blood-cerebrospinal fluid barrier (CSF), and/or infection through olfactory neurons (Ludlow et al. 2016). Several viruses target neurons which promote neuronal death and neuroinflammation. Microglial cells are the first responders during any pathogenic insult by activating the antiviral responses. However, uncontrolled/excessive microglial activation promotes loss of neuronal plasticity, synaptic termini elimination, demyelination of neurons, disruption of the BBB integrity, or neuronal death during a pathogen attack, which severely affects brain homeostasis. Often, neuroviral infections lead to encephalitis, encephalopathy, and meningitis that render the patient with long-term neurological sequelae or death (Das et al. 2008; Eggers et al. 2017; Figueiredo et al. 2019; de Andrade et al. 2021; Shigemoto-Mogami et al. 2018).

2 Role of Microglial Cells During Neuroviral Infections

Upon sensing the disturbances, microglial cells change either to the classic M1 phase or the alternative M2 phase. The classic M1 phase releases interferon and pro-inflammatory cytokines and chemokines to help in activating and linking the innate and adaptive responses and clearing the infection. During a pathological insult, the activated microglial cells express receptors like MHC-I/II, CD80, CD86, CD40, CD45, CD11b, CD54, and CD58 molecules for antigen presentation and activate T lymphocytes (Ford et al. 1996; Malone et al. 2008). The alternative M2 phase microglia initiate anti-inflammatory responses for repair and damage control postinfection, thereby maintaining homeostasis (Fig. 19.2).

The neurotropic virus has been listed in Table 19.1. Patients infected with the Japanese encephalitis virus (JEV) either suffer from long-term neurological sequelae or die due to acute encephalitis. The JEV and Zika virus (ZIKV) enter into the CNS via the "Trojan horse mechanism," similar to the human immunodeficiency virus-1 (HIV-1) (Persidsky et al. 1997; Xu et al. 2021). HIV-1-mediated neuropathy has been reported in almost 50% of patients. The generation of pro-inflammatory responses through secretion of interferons and formation of microglial nodules near and around infected neurons and astrocytes contribute to the neuropathogenesis (Alirezaei et al. 2008; Churchill et al. 2009). Opportunistic infections are linked with HIV-1 infections, including tuberculosis meningitis, cytomegalovirus (CMV) infection, cryptococcal meningitis, cerebral toxoplasmosis, etc. (Tan et al. 2012). Dengue virus (DENV)-mediated acute encephalitis in mice results in the loss of

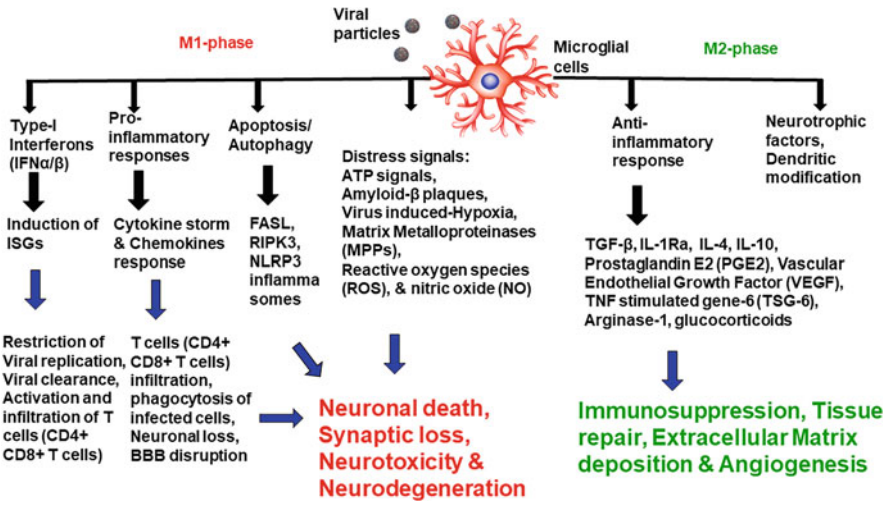

Fig. 19.2 Role of microglial cells in the pathophysiology of viral Infections The microglial cells are brain macrophages that polarize to an M1 or M2 phenotype depending upon the external stimuli. The M1 phase produces an antiviral and pro-inflammatory response upon viral detection to clear the infection. However, the M2 phase microglia lower inflammation by releasing anti-inflammatory responses which help in tissue repair and regeneration

hippocampus neurons, which affects learning ability and memory (Tsai et al. 2016). The dysregulation of microglial cells not only contributes to long-term sequelae but promotes neurodegenerative diseases like Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS) (Eggers et al. 2017). Neurological complications have been reported in patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Asadi-Pooya and Simani 2020; Gonçalves de Andrade et al. 2021). Infection with alpha-viruses leads to high morbidity and post-infection long-term neurological sequelae including neuropsychological changes and intellectual disabilities (Ronca et al. 2016).

Table 19.1 List of neurotropic viruses and neurological manifestations

Family	Virus	Clinical presentation
Flaviviridae	Dengue virus (DENV)	Encephalitis, meningitis, stroke, and cerebellar hemorrhage
	Zika virus (ZIKV)	Guillain-Barré syndrome, microcephaly, congenital Zika syndrome (CZS), Lissencephaly resulting in mental retardation
	Japanese encephalitis virus (JEV)	Encephalitis or acute flaccid paralysis (AFP)
	West Nile virus (WNV)	Spinal motor neurons causing acute asymmetric flaccid paralysis
	Tick-borne encephalitis virus (TBEV)	Meningitis, encephalitis, and meningoencephalitis
Togaviridae	Venezuelan equine encephalitis virus (VEEV)	Convulsions, somnolence, confusion, photophobia, coma, intellectual disability, and emotional instability/behavioral changes
	Western equine encephalitis virus (WEEV)	Visual disturbances, spastic paresis
	Eastern equine encephalitis virus (EEEV)	Seizures, paralysis, intellectual disability, and behavioral changes
	Chikungunya virus (CHIKV)	Alteration in consciousness, cranial nerve deficits, seizures, hemi/paraparesis, and involuntary movements
Retroviridae	Human immunodeficiency virus-1 (HIV-1)	HIV-1-associated dementia, HIV-1-associated neuromuscular disease, multifocal leukoencephalopathy (PML), and cytomegalovirus (CMV) infection
	Human T-lymphotropic virus (HTLV)	Myelopathy/tropical spastic paraparesis, polyneuropathies, motor neuron disease like myasthenia gravis-like syndrome
Rhabdoviridae	Rabies virus (RABV)	Acute flaccid paralysis
	Chandipura virus (CHPV)	Encephalitis, seizures
	Vesicular stomatitis virus (VSV)	Encephalitis, neurodegeneration

(continued)

Family	Virus	Clinical presentation
Picornaviridae	Theiler's murine encephalomyelitis virus (TMEV)	Encephalitis
	Encephalomyocarditis virus (EMCV)	Encephalitis, hind limb paralysis, and demyelination
Coronaviridae	Severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1)	Stroke, neuromuscular disorder, encephalopathy, encephalitis, and meningitis
	Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)	Seizures, meningoencephalitis, GBS, acute myelitis, posterior reversible encephalopathy syndrome (PRES), anosmia, and dysgeusia
	Middle East respiratory syndrome coronavirus (MERS-CoV)	Acute disseminated encephalomyelitis (ADEM)
Herpesviridae	Herpes simplex virus-1 (HSV-1)	Encephalitis
	Cytomegalovirus (CMV)	Acute inflammatory demyelinating polyneuropathy, myeloradiculopathy, mononeuritis multiplex, distal peripheral neuropathy
	Human herpes virus-6 (HHV-6)	Encephalitis cerebellitis and rhombencephalitis, febrile seizures
	Varicella-zoster virus (VZV)	Acute inflammatory demyelinating polyneuropathy, postherpetic neuralgia, motor neuropathy, cranial neuropathy, myeloradiculitis
	Epstein-Barr virus (EBV)	Acute inflammatory demyelinating polyneuropathy, myeloradiculitis, encephalomyeloradiculitis
Bunyaviridae	La Crosse virus (LACV)	Aseptic meningitis, encephalitis, or acute flaccid paralysis
Arenaviridae	Lymphocytic choriomeningitis virus (LCMV)	Fatal meningitis, sensory neuropathy
Paramyxoviridae	Nipah virus (NiV)	Encephalitis followed by long-term neurological and functional morbidity
	Hendra virus (HeV)	Acute and relapsing encephalitis

2.1 Antiviral Roles of Microglial Cells During Neuroviral Infections

Microglial cells express various types of pathogen recognition receptors (PRRs) that recognize a specific pattern or sequences present in pathogens known as pathogen-associated molecular patterns (PAMPs) (Table 19.2). The PRRs include Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), C-type

Table 19.2 The different PRRs present in microglial cells

PRRs	Location	Types
TLRs	Cell surface	TLR1, TLR2, TLR4, TLR5, TLR6, TLR10
	Endosome	TLR3, TLR7, TLR8, TLR9
CLRs	Cell surface	DC-SIGN, MINCLE, CLEC9a, CLEC5a, DEC205
RLRs	Cytoplasm	RIG-1, MDA5, LGP2
NLRs	Cytoplasm	CIITA, NAIPs, NOD1, NOD2, NLRC4, NLRX1, NLRC3, NLRC5, NLRP1, NLRP2-9, NLRP11-14, NLRP10
ALRs	Cytoplasm	AIM-2, IFI16

lectin receptors (CLRs), and AIM-2-like receptors (ALRs), which mediate the innate immune responses against viral infections. Some of these PRRs are expressed on the cell surface (TLRs, CLRs), while others are endogenously located in the endosome (TLRs) and the cytoplasm (RLRs, NLRs) to recognize both single- and double-stranded RNA and DNA viruses (Goubau et al. 2013; Lee et al. 2019).

The antiviral response of microglial cells during viral infections is mediated through the activation and secretion of interferons (Wheeler et al. 2018; Daffis, Samuel, Suthar, Gale, & Diamond, 2008; Drokhlyansky et al. 2017; Han et al. 2014; Priya et al. 2014; Town et al. 2006; Verma and Bharti 2017; Wang et al. 2004). The PRRs present on microglial cells help in initiating both pro-inflammatory and antiviral responses upon viral recognition. In addition, the activated microglial cells secrete cytokines (TNFα, interleukins, and interferons), chemokines, and chemoattractants to lure the peripheral immune cells to the place of infections.

The IFNα/β responses are initiated by Toll-like receptors (TLRs) present on the cell surface and in the endosomes. There are several TLRs that recognize RNA/DNA viruses (TLR3, TLR4, TLR6, TLR7, TLR8, TLR9, and TLR10) (Akira et al. 2006; Singh et al. 2021). The typical TLR structure comprises of a leucine-rich repeat (LRR) above the cell surface, the transmembrane helix embedded in the plasma membrane, and the intercellular TIR domain facing the cytoplasm, which interacts with the other signaling molecules (Akira et al. 2006). The different adaptor proteins that bind to the TIR domain of TLRs to initiate downstream signaling include TRIF-related adaptor molecule (TRAM/TICAM2) and TIR domain-containing adaptor-inducing interferon-β (TRIF), myeloid differentiation primary response protein 88 (MyD88), and TIR domain-containing adaptor protein (TIRAP or MAL) (Akira and Takeda 2004). TLR3 is present on the endosome, while TLR4 is present on the cell membrane, which is internalized into the endosome upon ligand binding. The TRAM and TRIF bind to the TLR3/TLR4 TIR domains and phosphorylate and activate the interferon response factor 3 (IRF3) and IRF3-related genes to induce the IFN-β response (Doyle et al. 2002; Kawai and Akira 2010; Oshiumi et al. 2003; Yamamoto et al. 2003). TLR7, TLR8, and TLR9 are present on the endosomes and initiate the IFN-β response by the binding of MyD88 to the TLRs (Kawai et al. 2004). MyD88 is an adaptor protein that activates the IRF7 transcription, which leads to an IFN-β response (Honda et al. 2005). TLR3 activates both the interferon

and pro-inflammatory responses via IRF3 and NF-κB pathways (Alexopoulou et al. 2001). TLR3 restricts herpes virus infection-1 (HSV-1) replication in the CNS via type-I IFN response (Conrady et al. 2010). TLR3-deficient mice succumb to West Nile virus (WNV) infection due to an excessive viral load and neuroinflammation (Daffis et al. 2008). TLR3 in combination with TLR4 recognizes the JEV. JEV infection in TLR3-deficient mice results to an increased inflammation in brain. However, TLR4-deficient mice have mild JEV-mediated inflammation (Han et al. 2014). The mutations in TLR3 genes increase HSV-1-mediated encephalitis and have been shown to promote latency (Lellouch-Tubiana et al. 2000; Lim et al. 2014). The TLR3-TRIF signaling axis was highly dampened in HSV-1-infected patients (Andersen et al. 2015). In vivo and in vitro studies have suggested the mutation in IRF3, which dampened the interferon response against HSV-1 and resulted in encephalitis (Andersen et al. 2015; Menachery et al. 2010). TLR7 has been reported to contribute to the production of type-I IFN response in mice infected with JEV (Nazmi et al. 2014). TLR7 and MyD88 knockout mice contribute to high mortality in mice infected with WNV. The suppression of interleukin-23 (IL-23) via TLR7 fails to recruit T cells that contribute to viral load (T. Town et al. 2009). The JEV is recognized by TLR7/TLR8 and activates the type-I IFN via the RIG-1-IRF3 axis (Chang et al. 2006).

The IFN-I is recognized by the IFNAR present on the cell surface which initiates the dimerization of receptor tyrosine kinases (RTKs) and non-receptor tyrosine-protein kinase 2 (JAK2 and TYK2). The RTKs induce the hetero-dimerization of STAT1/STAT2 along with IRF9, which forms a transcriptional factor complex (ISGF3). The complex translocates to the nucleus and binds to the promoter region of the interferon-stimulated response element (ISRE) and induces the expression of several interferon-stimulated genes (ISGs). The ISGs help in blocking viral replication, translation, transcription, assembly, and exocytosis from infected cells (Drokhlyansky et al. 2017; Fensterl et al. 2012; Platanias 2005; Schreiber and Piehler 2015; Sharma et al. 2015). The in vivo mouse model of vesicular stomatitis virus (VSV) has shown that microglial cells are activated through IFNAR signaling of infected neurons and astrocytes. The VSV-induced microglial activation forms an innate barrier in the olfactory bulb and restricts the viral spread to the CNS (Chhatbar et al. 2018). The VSV and encephalomyocarditis virus (EMCV) infection induced the expression of IFNβ, ISG54, and ISG56 via IFNAR signaling in the CNS of mice. However, only ISG54 is indispensable for clearing the VSV infection in the CNS, and no effect was seen during EMCV infection (Fensterl et al. 2012). JEV infection in microglial cells suppresses antiviral responses via targeting STAT-1 and antiviral genes like ISG54 and ISG56 (Sharma et al. 2015). The ZIKV-NS5 protein antagonizes type-I interferon signaling by degrading the STAT-2 proteins (Grant et al. 2016). Upon virus internalization, the cytosolic sensors recognized the ss/dsRNA viruses via retinoic acid-inducible gene I (RIG-1) and melanoma differentiation-associated protein 5 (MDA-5) and DNA viruses by cytosolic cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING). The RIG-1 and MDA-5 are helicases that induce the IFNα/β response and pro-inflammatory cytokines via mitochondrial antiviral signaling protein/caspase

activation recruitment domain adaptors inducing IFN-β (MAVS/CARDIF) or IFN-β promoter stimulator 1 (IPS-1), TANK-binding kinase 1 (TBK-1), and IKKε (Fitzgerald et al. 2003; ten Oever et al. 2007). The mouse model of EMCV infection has shown the induction of an antiviral response via the MDA-5-IPS-1-TBK-1 axis (Kato et al. 2006). Moreover, VSV infections are recognized by MDA-5 and induce an antiviral response in murine microglial cells (Furr et al. 2008). Zika virus (ZIKV) infection in microglial cells has been shown to increase the expression of IFN-α/β (Diop et al. 2018). The STING-mediated phosphorylation of IRF3 results in the production of the IFN-β response (Furr and Marriott 2012; Sun et al. 2013). HSV-1 has been reported to induce type-I interferon via the cGAS-STING pathway in mice model. The suppression of the cGAS-STING axis increases the viral load in midbrain, hypothalamus, and preoptic nerve area (Reinert et al. 2016).

The mouse microglial cells infected with mouse hepatitis virus (MHV) induce a type-I IFN response. The depletion of the microglial population by using colony-stimulating factor 1 receptor (CSF1R) inhibitors contributes to the development of encephalitis and leads to death (Wheeler et al. 2018). The exogenous treatment by IFN-α and IFN-β restricts MHV replication in in-vivo system (Cervantes-Barragan et al. 2006). It has been reported that La Crosse virus-infected (LACV) mouse microglial cells induce the IFN-β response (Kallfass et al. 2012). The exogenous treatment of IFN-β protects newborn mice from lethal HSV-1 infection (Giraldo et al. 2020). The mouse infected with VSV changes the microglial morphology and produces a type-I IFN response, which restricts viral replication in the brain parenchyma. In addition, the uninfected nearby microglial cells induce the expression of IRF7 to activate innate immune response and restrict VSV transsynaptic spread (Drokhlyansky et al. 2017). Rabies virus (RABV) infection mounts the type-I interferon response via the RIG-1-IRF3 or STATs or IRF7 pathway in microglia, neurons, and astrocytes (Zhao et al. 2013).

2.2 Pro-Inflammatory Response of Microglial Cells During Neuroviral Infection

The pro-inflammatory responses are initiated by PRRs including TLRs, NLRs, TNFR1, IL-1R1, and triggering receptor expressed on myeloid cells (TREMs). The receptor-ligand binding activates the downstream signaling cascade which leads to the production of pro-inflammatory responses (IL-6, IL-12, and IFN-γ, etc.) and chemokines (CCR2, CXCL1, CXCL10, RANTES, MCP1, IL-18) and generation of reactive oxygen species (ROS), cyclooxygenase-2 (COX-2), and nitric oxide synthase-2 (NOS-2). These responses are mediated by the activation of MyD88-dependent or TRIF-dependent NF-κB response, caspases, MAPK, and JNK family that activate AP-1 transcriptional factors (NOS-2 and COX-2) and activation of NLRP3 inflammasomes, Pellino proteins deubiquitinated by RIPK3 proteins, and Pellino proteins mediating pro-inflammatory responses (Rastogi and Singh 2020a; Rodríguez-Gómez et al. 2020).

The TLR2-deficient mouse model of HSV-1 has been shown to protect against lethal encephalitis by suppressing the secretion of IL-6 and MCP-1 in the brain resulting in reduced mortality (Kurt-Jones et al. 2004). The synergistic role of TLR2 and TLR9 has been reported in the mice model of HSV-1. The synergistic role of TLR2 and TLR9 has been reported in the mouse model of HSV-1. The knockout TLR2/TLR9 mice succumb to lethal encephalitis due to the lack of TNF-α and CXCL9 in the brain (Sørensen et al. 2008). TLR3 recognizes WNV during the replication cycle, compromises the blood-brain barrier (BBB) via TNFα1R, and contributes to lethal encephalitis. However, knockout of TLR3 protects mice from WNV-mediated lethal encephalitis and cytokine storm (Alexopoulou et al. 2001; Wang et al. 2004).

JEV infection in microglial cells leads to the expression of IL-1β and IL-18 and mediates neuronal cell death in a bystander fashion (Das et al. 2008). JEV and WNV infection elevate the expression of iNOS, COX-2, CXCL10, CXCL1, CCL2, CCL3, CCL5, TNF-α, TNF-related apoptosis-inducing ligand (TRAIL), IL-6, IL-1β, and MCP-1 in microglial cells and contribute to neuronal loss (Chen et al. 2010; Ghoshal et al. 2007; Quick et al. 2014). Treatment with minocycline readily reduces the expression of ROS, CCL2, 5, and IL-6 and helps in reducing neuroinflammation in both JEV and WNV (M. K. Mishra et al. 2009; Quick et al. 2014). ZIKV and ZIKV-NS1 protein both contribute to ROS generation in astrocytes and brain microvascular endothelial cells (BMVECs), which promotes microglial activation (Ledur et al. 2020; Rastogi and Singh 2020b). The HIV-1 Tat protein mediates microglial activation by inducing NADPH-dependent ROS activation and by suppressing the tumor necrosis factor receptor-associated factor-3 (TRAF3) proteins (Barger et al. 2007; R. Mishra et al. 2012). The HSV-1 infection secretes TNF-α, IFN-γ, and nitric oxide (NO) to control viral replication in the CNS (Khanna et al. 2004). Chandipura virus (CHPV) infection activates the microglial cells via iNOS, COX-2, NO, and ROS generation in the brain, which mediates the neuroinflammation and bystander killing of neurons (A. K. Verma et al. 2016). The microglial cells are permissive to human herpes virus-6 (HHV-6) infection and cause acute encephalopathy which leads to the production of IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, and soluble TNFR1, as detected in the cerebrospinal fluid (CSF) sample (Bortolotti et al. 2019; Ichiyama et al. 2009). The infection of Theiler's murine encephalomyelitis virus (TMEV) in microglial cells expresses genes like IL-1β, TNF-α, IL-6, iNOS, and macrophage inflammatory protein-1 alpha (MIP1-α/CCL3) that contribute to neuroinflammation (DePaula-Silva et al. 2019; Olson et al. 2001). The microglia and type I astrocytes are the main sources of cytokine storm in murine coronavirus infection (MHV-A59 infection), secreting IL-6, TNF-α, and IFNs and forming a perivascular glymphatic system near endothelial cells (Lavi and Cong 2020). The mouse microglial cells infected with RABV activate the microglial cells and produce interferon-stimulated genes (ISG15, ISG20, OASL1, OASL2, IFIT2, IRF7, and IFIT203), chemokines (CCL5, CXCL10, and CCRL2), and inflammatory cytokines, IL-6, TNF-α, IL-12, and IL-5 (Solanki et al. 2009; Zhao et al. 2013). The Venezuelan equine encephalitis virus (VEEV) infection in microglial cells results in mitochondrial dysfunction and production of IL-1α and IL-1β, IL-6, and IL-8

(Keck et al. 2018). The fetus infected with DENV presented with a robust cytokine storm (RANTES, monocyte chemoattractant protein-1 (MCP-1/CCL2), IFN-γ, and vascular endothelial growth factor (VEGF)) and DENV-NS3 antigen in microglial cells (Nunes et al. 2019). HIV-1 Tat protein has been implicated in microglial activation and production of pro-inflammatory responses via NLRC5-NF-κB and MEPC2-STAT3 or by activating the NLRP3 inflammasome pathway (Chivero et al. 2017; Periyasamy et al. 2019; Periyasamy et al. 2018; Wallet et al., 2019). JEV activates the NLRP3 inflammasome via a caspase-dependent pathway to mount pro-inflammatory responses from activated microglia (Kaushik et al. 2012). ZIKV-NS5 initiates the generation of the inflammasome complex in ZIKV-infected cells via caspase-1, which leads to the production of IL-1β and ROS generation (He et al. 2018; Tricarico et al. 2017). The HHV-6 infection contributes to the development of Alzheimer's disease by increasing the expression of TREM2 on microglial cells, promoting neuroinflammation via the PI3K/AKT/NF-κB axis, and TREM2-apolipoprotein E4 (ApoE4)-mediated IL-1β expression (Bortolotti et al. 2019).

Glutamate (Glu) is an excitatory neurotransmitter released during neurotransmission. Viruses and viral proteins have been identified which interfere with synaptic transmission, thereby hindering cell communication and promoting neurotoxicity. The N-methyl-D-aspartate (NMDA) is a Glu receptor present in microglia that upon overstimulation mediates neuronal damage (Zhou and Danbolt 2014). HIV-1 infection mediates the Glu release from the infected neurons and activates the microglial cells, which further mediates the HIV-1-associated neurocognitive disorders (HAND) and promotes latency in the CNS (Gorska and Eugenin 2020). The JEV-infected microglial cells have been shown to secrete Glu which is sensed by NMDAR to mediate excitatory neurotoxicity and leads to neurodegeneration. The TNF-α-mediated microglial activation enhances the Glu release during JEV infection (Chen et al. 2012; Chen et al. 2018).

The ZIKV exploits the yolk sac microglial progenitor cells to disseminate the virus in the developing fetus (Xu et al. 2021). Moreover, the studies have shown that ZIKV is permissive to microglial cells and induces the expression of TNF-α, IL-6, IL-1β, and the chemokine receptor CX3CR1. CX3CR1 maintains the functional synaptic plasticity of neurons and signaling between microglial cells. CX3CR1 regulates axon outgrowth and signals synaptic pruning in the CNS. Further, its increase in CX3CR1 is neurotoxic to the CNS (Diop et al. 2018; Pagani et al. 2015). The microglia express CX3CR1, which mediates the cell-to-cell transmission of JEV in the CNS (He et al. 2018). The CXCR3-deficient mice infected with HSV-1 reduced the infiltration of T cells and suppressed the activation of microglial cells (Zimmermann et al. 2017). The blocking of CCR2 by RS102895 during JEV infection reduces the pro-inflammatory response (TNF-α and IFN-γ) and NO in murine microglial cells (Singh et al. 2020). The TMEV mouse model has shown that the activated microglia secrete CCL2, which is the ligand for the CCR2 receptor present on peripheral monocytes. The CCL2/CCR2 cross talk mediates monocyte infiltration into the CNS to mediate the degradation of the hippocampus region. This led to the development of neurodegeneration and seizures in mice (Käufer et al. 2018). CXCR4 and CCR5 are expressed on microglial cells and have been

implicated in HIV-1 internalization promoting neuronal degradation (Cartier et al. 2005). The CCR2/CCR5 receptor mediates the infiltration of peripheral monocytes into the CNS during HIV-1 infection. The transmigration of peripheral monocytes is mediated by the junctional proteins, JAM-A, and ALCAM during HIV-1 infection (Williams et al. 2014).

2.3 Microglial Cells Affect Adaptive Immune Response During Neuroviral Infection

Studies have shown that microglial cells act as a potential reservoir for JEV, TMEV, and HIV-1 infection and contribute to JEV-mediated long-term neurological sequelae, TMEV-induced demyelinating disease (TMEV-IDD), and HIV-1-associated neurocognitive disorders (HAND) (Olson et al. 2001; Ravi et al. 1993; Sutherland and Brew 2018; Thongtan et al. 2010; Wong et al. 2019). The activated microglial cell population persists latency in patients infected with HSV-1 for more than 12 months. The major histocompatibility complex (MHC) class I and class II are expressed on the surface of all antigen-presenting cells, including microglial cells. CD40 and CD86 are the co-stimulatory molecules that help microglial cells present antigenic peptides to CD8+ and CD4+ T cells. The activated microglial cells recruit CD4+ and CD8+ T cells and localize near the infected neurons and phagocytose the infected neurons (Cagnin et al. 2001; Hüfner et al. 2006; Theil et al. 2003). The mouse model of WNV has shown that activated microglial cells release TNF-α, which mediates the infiltration of CD8+ T cells that help clear the virus while, on the other hand, promoting neuronal damage by inducing the expression of IFN-γ level (Shrestha et al. 2008). The IL-1R signaling attracts CD4+ T cells into the CNS to clear the WNV infection in the mouse mode (Durrant et al. 2013). The RABV-infected microglial cells secrete nitric oxide (NO) which allows the infiltration of CD4+ and CD8+ T cells to promote neuronal apoptosis and orchestrate neuroinflammation (Madhu et al. 2016). Experiments on MHV-infected mice with depleted microglial cells (CSF1R inhibitor) have shown that the number of CD4+ T cells declines while CD8+ T cells increase. The depletion of microglial cells resulted in the reduction of CD4+ T cell-mediated IFN-γ response and increased viral load in the CNS (Wheeler et al. 2018). Similar observations were made in the TMEV-infected mice, where the microglial cell depletion reduced the CD4+ T cell infiltration. Besides, an increase in IL-6 and IL-10 expression has been observed, which is possibly responsible for the increase in seizures, neuroinflammation, and neurodegeneration (Waltl et al. 2018). The depletion of the microglial cell population during DENV infection contributes to an increase in viral load, neuropathy, and death. The lack of antiviral response from microglial cells impedes the infiltration of cytotoxic T cells (CTLs-CD8 + T cells) and promotes DENV-mediated direct neurocytotoxic effect on the neurons of the hippocampus region, which is linked with the learning and memory process (Tsai et al. 2016). The depletion of microglial cells during WNV infection has shown that microglia are required for local reactivation of T cells. The study has shown a decrease in co-stimulatory signals and inflammatory

responses in the absence of microglial cells, which eventually leads to increased viral load and neuronal death (Funk and Klein 2019). The infiltrated CD8+ T cells post WNV and ZIKV infection produce IFN-γ that activates the microglial cells and contributes to presynaptic termini elimination in WNV mice while complete loss of neurons and presynaptic termini in ZIKV recovered mice. These findings indicate that T cell response to microglia promotes cognitive dysfunction postinfection (Garber et al. 2019). The infiltrating T cell population post-RABV infection contributes to neuroinflammation and mediates BBB dysfunction via Collapsing Response Mediator Protein 2 (CRMP2) (Vuaillat et al. 2008). The cross-presentation is the ability to present exogenous antigens on class I MHC which are normally presented on class II MHC to CD8+ T cells. Dendritic cells are highly efficient in cross-presenting cells in the CNS (Embgenbroich and Burgdorf 2018). However, microglia have also been reported to cross-present antigens during viral infection (Beauvillain et al. 2008). The VSV-infected mouse model has shown that uninfected microglial cells exhibit cross-presentation from an infected neuron to antiviral CD8+ T cells which non-cytolytically clear the infected neurons (Moseman et al. 2020). The mouse model of lymphocytic choriomeningitis virus (LCMV) demonstrated that therapeutically administered T cells clear the infection without breaching the BBB or causing any neuronal damage. The CD8+ and CD4+ T cells directly interact with CD11c + microglial cells to induce STAT-1 to activate the interferon response (Herz et al. 2015).

The WNV mouse model has shown that the infected neurons activate the microglial cells, promote complement activation (C1qa, C3, C4b), and phagocytose the presynaptic neurons, which leads to memory loss (Vasek et al. 2016). ZIKV-infected neurons and neural progenitor cells (NPCs) secrete TNF-α that activates the microglial cells and promotes complement (C3 proteins) activation in the mice brain. Microglial activation leads to synaptic loss which impairs memory in mice (Figueiredo et al. 2019). The microglial cells infected with TMEV express genes involved in complement activation like C1ra, C2, C4b, and Masp1 that promote neuroinflammation (DePaula-Silva et al. 2019).

3 Microglia Perturb Cell Death Pathways During Viral Infection

To promote their survival and propagation inside the host, viruses target cell death pathways. Autophagy is a regulated, multistep process of intracellular degradation that clears invading pathogens and maintains cellular homeostasis. During viral infections, autophagy plays a key role in cellular immunity. The viruses may either take over the cell's autophagy machinery or express proteins to help them evade autophagy and promote their replication. As a part of the antiviral response, autophagy may (a) regulate cell survival or inflammation; (b) promote pathogen recognition, antigen presentation, or inflammatory response; and (c) promote degradation of viruses (Choi et al. 2018).

DENV activates autophagy in cells to promote its replication (Acharya et al. 2019). Inhibition of autophagy decreases the number of viral RNA and extracellular virions (Mateo et al. 2013). Activation of autophagy restricts Sindbis virus replication and transmission (Orvedahl et al. 2010). In contrast, HSV-1 and HIV-1 promote replication by inhibiting autophagy (Killian 2012; O'Connell and Liang 2016).

ZIKV infection in human skin cells results in the formation of autophagosomes and promotes viral replication (Hamel et al. 2015). NF-kB activation occurs in ZIKV-infected drosophila, which promotes dSTING-mediated activation of autophagy and inhibits ZIKV replication (Liu et al. 2018). Deficiency of the autophagy gene Atg16l1, in mice, inhibited autolysosome maturation and prevented vertical transmission of ZIKV (Cao et al. 2017). ZIKV infection leads to induction of autophagy and promotes autophagophore formation in BV2 microglia. This helps the host to resist pathogens and accelerates ZIKV clearance from microglia and macrophages (Y. Huang et al. 2020).

Apoptosis is a programmed series of perturbations which occur in the cell and result in cell death. It involves the activation of cysteine proteases, caspases which cause proteolysis of several proteins. There are two routes to apoptosis: (1) the mitochondrial pathway, which is regulated by bcl-2 protein and involves a decrease in mitochondrial membrane potential and release of cytochrome c from mitochondria resulting in activation of caspase-9 and caspase-3, and (2) the death receptor pathway which responds to the signals initiated by the cell death ligands. It activates the FADD, resulting in activation of caspase-8, caspase-3, and caspase-7 (Kvansakul 2017).

In response to viral infections, apoptotic cell death may either clear the virus from the host cell or may be utilized by the viruses for their multiplication and progression of disease. HSV infection in murine microglia activates the TLR-2 and TNF-pathways, leading to perturbation of apoptotic genes in microglia and ultimately leading to cell death (Aravalli et al. 2007). Further, it was seen that HSV infection caused apoptosis in microglia and other immune cells in a cGAS-/STING-dependent manner in the brain microenvironment independently of IFN-1 (Reinert et al. 2021).

ZIKV infection is known to trigger apoptosis in human NPCs, leading to microcephaly (Z. He et al. 2020). ZIKV infection in microglia downregulates anti-apoptotic protein Bcl-2 and promotes apoptosis of microglia by a PARP-dependent caspase mechanism (Turpin et al. 2019). ZIKV utilizes the Tam receptor, Axl, for its entry into the cells, and this receptor plays a crucial role in ZIKV-mediated induction of apoptosis in microglial cells. It was seen that the ZIKV infection in the $Ifnar^{-/-}Axl^{-/-}$ mouse model caused reduced apoptosis as compared to the wild type (Hastings et al. 2019).

DENV infection in newborn mice caused fatal encephalitis within 10 days of infection by promoting apoptosis in the cortical and hippocampal regions of the mouse brain (Desprès et al. 1998). DENV2 infection in BV2 cells increases iNOS levels and produces oxidative stress leading to apoptosis in microglial cells (Jhan et al. 2017).

HIV-1 infection in human microglia leads to apoptosis in most of the cells, but a small population survives and becomes an HIV-1 reservoir. These cells have

upregulated expression levels of Bim, which is a pro-apoptotic negative regulator of Bcl-2. This prevents these reservoir cells from further apoptosis (Castellano et al. 2017). Microglia in HIV encephalitis patients are more susceptible to apoptosis. In these patients, HIV-1 infection upregulates the pro-apoptotic protein Bax and inactivates the anti-apoptotic proteins Bcl-2 and Bcl-x (Perfettini et al. 2005). In contrast, it is also seen that HIV infection in primary human microglial cells upregulates the expression levels of BAG3, leading to a reduction in HIV-1-mediated apoptosis of microglia (Rosati et al. 2009).

4 Other Roles of Microglia During Viral Infection

Microglia are the hub of the brain's antiviral network. Apart from the pathways discussed above, there are a few distinct mechanisms by which microglia sense the presence of viruses in the CNS and help in clearance. A few uncertain roles of microglia are discussed below, which would help in better understanding of microglia-mediated viral pathogenesis.

4.1 Microglial Activation During Virus-Induced Amyloid-β Plaque Formation

Amyloid-β (Aβ) protein is synthesized by the cleavage of mature amyloid precursor protein (APP) by β- and γ-secretase in the ER of neuronal cells. The Aβ peptide monomers assemble to form amyloid fibrils that form Aβ plaques (Chen et al. 2017). Under physiological state , microglial cells maintain the concentration of Aβ peptides, which in turn helps in maintaining the neuronal plasticity and memory. In addition, Aβ peptides are a component of innate immunity which provide antifungal and antibacterial activity in the CNS (Kumar et al. 2016). During viral infections, the concentration of Aβ peptides increases around the neurons. The high concentration of Aβ peptides leads to oxidative stress, changes in membrane permeability, synaptic dysfunction, reduction in mitochondrial ATP synthesis in neurons, and microglial activation (Brown et al. 2020; Chen et al. 2017).

Infection with *Herpesviridae* (HSV-1, HHV-6A, and HHV-6B) initiates the formation of Aβ plaques which help in entrapping the virus and, lead to microglial activation. Sustained microglial activation leads to neuronal death and failure to clear Aβ plaques from the CNS (Bourgade et al. 2016; Eimer et al. 2018). HIV-1 and its viral proteins (HIV-Tat and HIV-Gag) bind, secrete, and deposit thicker Aβ plaques. The thicker Aβ plaques result in increased neuronal damage and activate the microglial cells (Chai et al. 2017; Hategan et al. 2017). The infection with HIV-1 increases the propensity of Alzheimer's disease (AD) by depositing Aβ plaques (Ances et al. 2012; Ortega and Ances 2014). Microglia are very well studied in Aβ-mediated pathology in AD and HSV-1 infection. However, there is still a knowledge gap in understanding the involvement of microglia in Aβ plaque

development during HIV infection. Further studies focusing on the role of amyloid β in HIV infection will help in developing better therapeutics against HAND.

4.2 Roles of Microglial Purinergic Receptors During Viral Infection

ATP is an ubiquitous, metabolically active molecule. In the CNS, ATP is generated by microglia, neurons, and astrocytes (Cekic and Linden 2016). ATP binds to the purinergic family of receptors, P1 and P2 receptors. The P1 receptors bind to adenosine molecules and elicit a pro-inflammatory response, while the. P2 receptors (six P2XR homotrimers, four P2XR heterotrimers, and eight P2YR) bind to adenosine triphosphate and other purine and pyrimidine molecules and induce a pro-inflammatory response (Cekic and Linden 2016; Sperlágh and Illes 2007). Microglial cells express both P1 and P2 receptors. Microglia get activated in response to extracellularly released ATP (Sperlágh and Illes 2007). HSV-1-infected neuronal cells release ATP which activates the microglial cells via P2RY12 receptors. The $P2YR12^{-/-}$ mice showed a reduction of 50% in microglia recruitment around HSV-1-infected neurons. Microglial recruitment at the site of infection helps in curbing the infection in brain parenchyma by phagocytosis and leukocyte infiltration (Fekete et al. 2018). The pharmacological inhibition of purinergic receptors (P2X1, P2X7, and P2Y1) during HIV-1 infection suppresses viral entry and replication (Hazleton et al. 2012; Séror et al. 2011). HIV-1-mediated sensory neuropathy (HIV-SN) occurs in 60% of HIV-1-infected patients, which is marked by the loss of sensation in the nerve endings like the feet and fingers. The SNP-based study on HIV-1-infected patients has shown the association of sensory neuropathy with purinergic receptors (P2X4R and CAMKK2). The P2X4R mediates microglial activation and phagocytosis during HIV-1 infection (Gaff et al. 2018; Goullee et al. 2016). The SARS-CoV-2 has been implicated in Guillain-Barré syndrome (GBS), which is marked by microglial activation and cytokine storm mediated neuroinflammation. The release of ATP from infected cells activates the microglial cells via the P2X7R receptor. Therefore, the inhibition of the microglial receptor P2X7R might help treat SARS-CoV-2-mediated GBS patients (Simões and Bagatini 2021). In summary, the release of purines from infected cells has a central role in phagocytosis and activation of microglia via purinergic receptors. The use of purinergic receptor agonists or antagonists can be used as therapeutics against these viral infections.

4.3 Necroptosis and Microglia

Necroptosis is a host defense mechanism against infection mediated by receptor interacting protein kinase (RIPK1 or RIP1) and RIPK3 which leads to cell death. RIPK3 restricts the replication of HSV-1 and ZIKV in mice model. RIPK3 causes necroptosis in HSV-1 infected and ZIKV infected murine cells (Z. Huang

et al. 2015; B. P. Daniels et al. 2019). JEV infection in RIPK3$^{-/-}$ mice downregulates viral replication in neuronal cells by elevating the ISGs (Bian et al. 2020). WNV infection in microglial cells does not trigger RIPK3-mediated chemokine production (Brian P Daniels et al. 2017). RIPK3-induced signaling in microglia is not well understood and its role is under investigation.

5 Conclusion

Microglia are unique macrophages which play a central role in the maintenance of CNS homeostasis. Any aberration in CNS homeostasis activates microglial cells. Over the last few decades, our knowledge of microglial biology has developed vastly. Each year, new studies uncover new aspects of microglia's role in maintaining CNS homeostasis and immune regulation. Microglia are the first responders during infection or injury to the CNS. During viral infections, the infected microglial cells get activated and give rise to an antiviral immune response in the CNS. Neurotropic viral infection renders microglia overactive, which has a deleterious effect on the CNS. Overactivation of microglia contributes to disease severity and leads to long-term neurological sequelae like cognitive impairment, memory loss, seizures, and paralysis. Viral infections also affect the normal functioning of the microglial cells, leading to neuroinflammation. The mechanisms of most of these infections are yet to be understood. More research into microglial cell biology may help to uncover the role of microglia during neuroviral infections. Since most of our understanding is based on animal experiments and only a few findings have been confirmed in humans, more studies are needed on patient-derived microglial cells for a better understanding of pathophysiological mechanisms. This may add more information to our prevailing knowledge of microglial biology and help in the development of therapeutics and vaccines.

Acknowledgments Authors gratefully acknowledge the funding support received through the Institutions of Eminence Scheme (IoE-6031) of Banaras Hindu University, Varanasi, India, and Indian Council of Medical Research (2020/3602/CMB/ADHOC-BMS), Govt. of India, New Delhi.

References

Acharya B, Gyeltshen S, Chaijaroenkul W, Na-Bangchang K (2019) Significance of autophagy in dengue virus infection: a brief review. Am J Trop Med Hyg 100(4):783–790. https://doi.org/10.4269/ajtmh.18-0761

Akira S, Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4(7):499–511. https://doi.org/10.1038/nri1391

Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124(4):783–801. https://doi.org/10.1016/j.cell.2006.02.015

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-κB by toll-like receptor 3. Nature 413(6857):732–738. https://doi.org/10.1038/35099560

Alirezaei M, Kiosses WB, Fox HS (2008) Decreased neuronal autophagy in HIV dementia: A mechanism of indirect neurotoxicity. Autophagy 4(7):963–966. https://doi.org/10.4161/auto.6805

Ances, B. M., Benzinger, T. L., Christensen, J. J., Thomas, J., Venkat, R., Teshome, M., . . . Clifford, D. B. (2012). 11C-PiB imaging of human immunodeficiency virus-associated neurocognitive disorder. Arch Neurol, 69(1), 72–77. doi:https://doi.org/10.1001/archneurol.2011.761

Andersen LL, Mørk N, Reinert LS, Kofod-Olsen E, Narita R, Jørgensen SE et al (2015) Functional IRF3 deficiency in a patient with herpes simplex encephalitis. J Exp Med 212(9):1371–1379. https://doi.org/10.1084/jem.20142274

Gonçalves de Andrade, Elisa, Šimončičová, Eva, Carrier, Micaël, Vecchiarelli, Haley A., Robert, Marie-Ève, & Tremblay, Marie-Ève (2021). Microglia fighting for neurological and mental health: on the central nervous system frontline of COVID-19 pandemic. Front Cell Neurosci. 15(12). doi:https://doi.org/10.3389/fncel.2021.647378

Aravalli RN, Hu S, Lokensgard JR (2007) Toll-like receptor 2 signaling is a mediator of apoptosis in herpes simplex virus-infected microglia. J Neuroinflammation 4:11. https://doi.org/10.1186/1742-2094-4-11

Asadi-Pooya AA, Simani L (2020) Central nervous system manifestations of COVID-19: a systematic review. J Neurol Sci 413:116832. https://doi.org/10.1016/j.jns.2020.116832

Barger SW, Goodwin ME, Porter MM, Beggs ML (2007) Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. J Neurochem 101(5):1205–1213. https://doi.org/10.1111/j.1471-4159.2007.04487.x

Beauvillain C, Donnou S, Jarry U, Scotet M, Gascan H, Delneste Y et al (2008) Neonatal and adult microglia cross-present exogenous antigens. Glia 56(1):69–77. https://doi.org/10.1002/glia.20565

Bian P, Ye C, Zheng X, Luo C, Yang J, Li M et al (2020) RIPK3 promotes JEV replication in neurons via downregulation of IFI44L. J Front Microbiol 11:368

Bilimoria PM, Stevens B (2015) Microglia function during brain development: new insights from animal models. Brain Res 1617:7–17. https://doi.org/10.1016/j.brainres.2014.11.032

Bortolotti D, Gentili V, Rotola A, Caselli E, Rizzo R (2019) HHV-6A infection induces amyloid-beta expression and activation of microglial cells. Alzheimers Res Ther 11(1):104. https://doi.org/10.1186/s13195-019-0552-6

Bourgade K, Le Page A, Bocti C, Witkowski JM, Dupuis G, Frost EH, Fülöp T Jr (2016) Protective effect of amyloid-β peptides against herpes simplex virus-1 infection in a neuronal cell culture model. J Alzheimers Dis 50(4):1227–1241. https://doi.org/10.3233/jad-150652

Brown MR, Radford SE, Hewitt EW (2020) Modulation of β-amyloid fibril formation in Alzheimer's disease by microglia and infection. Front Mol Neurosci 13(228). https://doi.org/10.3389/fnmol.2020.609073

Cagnin A, Myers R, Gunn RN, Lawrence AD, Stevens T, Kreutzberg GW et al (2001) In vivo visualization of activated glia by[11C] (R)-PK11195-PET following herpes encephalitis reveals projected neuronal damage beyond the primary focal lesion. Brain 124(10):2014–2027

Cao B, Parnell LA, Diamond MS, Mysorekar IU (2017) Inhibition of autophagy limits vertical transmission of Zika virus in pregnant mice. J Exp Med 214(8):2303–2313

Cartier L, Hartley O, Dubois-Dauphin M, Krause KH (2005) Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. Brain Res Brain Res Rev 48(1):16–42. https://doi.org/10.1016/j.brainresrev.2004.07.021

Castellano P, Prevedel L, Eugenin EA (2017) HIV-infected macrophages and microglia that survive acute infection become viral reservoirs by a mechanism involving Bim. Sci Rep 7(1):12866. https://doi.org/10.1038/s41598-017-12758-w

Cekic C, Linden J (2016) Purinergic regulation of the immune system. Nat Rev Immunol 16(3):177–192. https://doi.org/10.1038/nri.2016.4

Cervantes-Barragan L, Züst R, Weber F, Spiegel M, Lang KS, Akira S et al (2006) Control of coronavirus infection through plasmacytoid dendritic-cell–derived type I interferon. Blood 109(3):1131–1137

Chai Q, Jovasevic V, Malikov V, Sabo Y, Morham S, Walsh D, Naghavi MH (2017) HIV-1 counteracts an innate restriction by amyloid precursor protein resulting in neurodegeneration. Nat Commun 8(1):1522. https://doi.org/10.1038/s41467-017-01795-8

Chang T-H, Liao C-L, Lin Y-L (2006) Flavivirus induces interferon-beta gene expression through a pathway involving RIG-I-dependent IRF-3 and PI3K-dependent NF-κB activation. Microbes Infect 8(1):157–171. https://doi.org/10.1016/j.micinf.2005.06.014

Chen CJ, Ou YC, Lin SY, Raung SL, Liao SL, Lai CY et al (2010) Glial activation involvement in neuronal death by Japanese encephalitis virus infection. J Gen Virol 91(Pt 4):1028–1037. https://doi.org/10.1099/vir.0.013565-0

Chen CJ, Ou YC, Chang CY, Pan HC, Liao SL, Chen SY et al (2012) Glutamate released by Japanese encephalitis virus-infected microglia involves TNF-α signaling and contributes to neuronal death. Glia 60(3):487–501. https://doi.org/10.1002/glia.22282

Chen G-f, Xu T-h, Yan Y, Zhou Y-r, Jiang Y, Melcher K, Xu HE (2017) Amyloid beta: structure, biology and structure-based therapeutic development. Acta Pharmacol Sin 38(9):1205–1235. https://doi.org/10.1038/aps.2017.28

Chen Z, Wang X, Ashraf U, Zheng B, Ye J, Zhou D et al (2018) Activation of neuronal N-methyl-d-aspartate receptor plays a pivotal role in Japanese encephalitis virus-induced neuronal cell damage. J Neuroinflammation 15(1):238. https://doi.org/10.1186/s12974-018-1280-8

Chhatbar C, Detje CN, Grabski E, Borst K, Spanier J, Ghita L et al (2018) Type I interferon receptor signaling of neurons and astrocytes regulates microglia activation during viral encephalitis. Cell Rep 25(1):118–129.e114. https://doi.org/10.1016/j.celrep.2018.09.003

Chivero ET, Guo ML, Periyasamy P, Liao K, Callen SE, Buch S (2017) HIV-1 tat primes and activates microglial NLRP3 inflammasome-mediated neuroinflammation. J Neurosci 37(13): 3599–3609. https://doi.org/10.1523/jneurosci.3045-16.2017

Choi Y, Bowman JW, Jung JU (2018) Autophagy during viral infection—a double-edged sword. Nat Rev Microbiol 16(6):341–354. https://doi.org/10.1038/s41579-018-0003-6

Churchill MJ, Wesselingh SL, Cowley D, Pardo CA, McArthur JC, Brew BJ, Gorry PR (2009) Extensive astrocyte infection is prominent in human immunodeficiency virus–associated dementia. Ann Neurol 66(2):253–258. https://doi.org/10.1002/ana.21697

Colonna M, Butovsky O (2017) Microglia function in the central nervous system during health and neurodegeneration. Annu Rev Immunol 35:441–468. https://doi.org/10.1146/annurev-immunol-051116-052358

Conrady CD, Drevets DA, Carr DJ (2010) Herpes simplex type I (HSV-1) infection of the nervous system: is an immune response a good thing? J Neuroimmunol 220(1–2):1–9. https://doi.org/10.1016/j.jneuroim.2009.09.013

Daffis S, Samuel MA, Suthar MS, Gale M Jr, Diamond MS (2008) Toll-like receptor 3 has a protective role against West Nile virus infection. J Virol 82(21):10349–10358. https://doi.org/10.1128/jvi.00935-08

Daniels BP, Snyder AG, Olsen TM, Orozco S, Oguin III, Thomas H, Tait SWG et al (2017) RIPK3 restricts viral pathogenesis via cell death-independent neuroinflammation. J Cell 169(2): 301–313.e311

Daniels BP, Kofman SB, Smith JR, Norris GT, Snyder AG, Kolb JP et al (2019) The nucleotide sensor ZBP1 and kinase RIPK3 induce the enzyme IRG1 to promote an antiviral metabolic state in neurons. Immunity 50(1):64–76.e64. https://doi.org/10.1016/j.immuni.2018.11.017

Das S, Mishra MK, Ghosh J, Basu A (2008) Japanese encephalitis virus infection induces IL-18 and IL-1beta in microglia and astrocytes: correlation with in vitro cytokine responsiveness of glial cells and subsequent neuronal death. J Neuroimmunol 195(1–2):60–72. https://doi.org/10.1016/j.jneuroim.2008.01.009

DePaula-Silva AB, Gorbea C, Doty DJ, Libbey JE, Sanchez JMS, Hanak TJ et al (2019) Differential transcriptional profiles identify microglial- and macrophage-specific gene markers

expressed during virus-induced neuroinflammation. J Neuroinflammation 16(1):152. https://doi.org/10.1186/s12974-019-1545-x

Derecki NC, Katzmarski N, Kipnis J, Meyer-Luehmann M (2014) Microglia as a critical player in both developmental and late-life CNS pathologies. Acta Neuropathol 128(3):333–345. https://doi.org/10.1007/s00401-014-1321-z

Desprès P, Frenkiel MP, Ceccaldi PE, Duarte Dos Santos C, Deubel V (1998) Apoptosis in the mouse central nervous system in response to infection with mouse-neurovirulent dengue viruses. J Virol 72(1):823–829. https://doi.org/10.1128/jvi.72.1.823-829.1998

Diop F, Vial T, Ferraris P, Wichit S, Bengue M, Hamel R et al (2018) Zika virus infection modulates the metabolomic profile of microglial cells. PLoS One 13(10):e0206093. https://doi.org/10.1371/journal.pone.0206093

Doyle SE, Vaidya SA, O'Connell R, Dadgostar H, Dempsey PW, Wu T-T et al (2002) IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity 17(3):251–263. https://doi.org/10.1016/S1074-7613(02)00390-4

Drokhlyansky E, Göz Aytürk D, Soh TK, Chrenek R, O'Loughlin E, Madore C et al (2017) The brain parenchyma has a type I interferon response that can limit virus spread. Proc Natl Acad Sci U S A 114(1):E95–e104. https://doi.org/10.1073/pnas.1618157114

Durrant DM, Robinette ML, Klein RS (2013) IL-1R1 is required for dendritic cell-mediated T cell reactivation within the CNS during West Nile virus encephalitis. J Exp Med 210(3):503–516. https://doi.org/10.1084/jem.20121897

Eggers C, Arendt G, Hahn K, Husstedt IW, Maschke M, Neuen-Jacob E et al (2017) HIV-1-associated neurocognitive disorder: epidemiology, pathogenesis, diagnosis, and treatment. J Neurol 264(8):1715–1727. https://doi.org/10.1007/s00415-017-8503-2

Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, Rodriguez AS, Mitchell T, Washicosky KJ et al (2018) Alzheimer's disease-associated β-amyloid is rapidly seeded by herpesviridae to protect against brain infection. Neuron 99(1):56–63.e53. https://doi.org/10.1016/j.neuron.2018.06.030

Embgenbroich M, Burgdorf S (2018) Current concepts of antigen cross-presentation. Front Immunol 9(1643). https://doi.org/10.3389/fimmu.2018.01643

Fekete R, Cserép C, Lénárt N, Tóth K, Orsolits B, Martinecz B et al (2018) Microglia control the spread of neurotropic virus infection via P2Y12 signalling and recruit monocytes through P2Y12-independent mechanisms. Acta Neuropathol 136(3):461–482. https://doi.org/10.1007/s00401-018-1885-0

Fensterl V, Wetzel JL, Ramachandran S, Ogino T, Stohlman SA, Bergmann CC et al (2012) Interferon-induced Ifit2/ISG54 protects mice from lethal VSV neuropathogenesis. PLoS Pathog 8(5):e1002712. https://doi.org/10.1371/journal.ppat.1002712

Figueiredo CP, Barros-Aragão FGQ, Neris RLS, Frost PS, Soares C, Souza INO et al (2019) Zika virus replicates in adult human brain tissue and impairs synapses and memory in mice. Nat Commun 10(1):3890. https://doi.org/10.1038/s41467-019-11866-7

Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT et al (2003) IKKε and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol 4(5):491–496. https://doi.org/10.1038/ni921

Ford AL, Foulcher E, Lemckert FA, Sedgwick JD (1996) Microglia induce CD4 T lymphocyte final effector function and death. J Exp Med 184(5):1737–1745. https://doi.org/10.1084/jem.184.5.1737

Funk KE, Klein RS (2019) CSF1R antagonism limits local restimulation of antiviral CD8+ T cells during viral encephalitis. J Neuroinflammation 16(1):22. https://doi.org/10.1186/s12974-019-1397-4

Furr S, Marriott I (2012) Viral CNS infections: role of glial pattern recognition receptors in neuroinflammation. Front Microbiol 3(201). https://doi.org/10.3389/fmicb.2012.00201

Furr SR, Chauhan VS, Sterka D Jr, Grdzelishvili V, Marriott I (2008) Characterization of retinoic acid-inducible gene-I expression in primary murine glia following exposure to vesicular stomatitis virus. J Neurovirol 14(6):503–513. https://doi.org/10.1080/13550280802337217

Gaff J, Halstrom S, Temple SEL, Baltic S, Kamerman P, Price P (2018) Polymorphisms in P2X4R and CAMKK2 may affect TNFα production: implications for a role in HIV-associated sensory neuropathy. Hum Immunol 79(4):224–227. https://doi.org/10.1016/j.humimm.2018.02.002

Garber C, Soung A, Vollmer LL, Kanmogne M, Last A, Brown J, Klein RS (2019) T cells promote microglia-mediated synaptic elimination and cognitive dysfunction during recovery from neuropathogenic flaviviruses. Nat Neurosci 22(8):1276–1288. https://doi.org/10.1038/s41593-019-0427-y

Ghoshal A, Das S, Ghosh S, Mishra MK, Sharma V, Koli P et al (2007) Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis. Glia 55(5): 483–496. https://doi.org/10.1002/glia.20474

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S et al (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330(6005):841–845. https://doi.org/10.1126/science.1194637

Giraldo D, Wilcox DR, Longnecker R (2020) The innate immune response to herpes simplex virus 1 infection is dampened in the newborn brain and can be modulated by exogenous interferon beta to improve survival. mBio 11(3). https://doi.org/10.1128/mBio.00921-20

Gorska AM, Eugenin EA (2020) The glutamate system as a crucial regulator of CNS toxicity and survival of HIV. Reservoirs 10(261). https://doi.org/10.3389/fcimb.2020.00261

Goubau D, Deddouche S, Reis e Sousa C (2013) Cytosolic sensing of viruses. Immunity 38(5): 855–869. https://doi.org/10.1016/j.immuni.2013.05.007

Goullee H, Wadley AL, Cherry CL, Allcock RJ, Black M, Kamerman PR, Price P (2016) Polymorphisms in CAMKK2 may predict sensory neuropathy in African HIV patients. J Neurovirol 22(4):508–517. https://doi.org/10.1007/s13365-015-0421-4

Grant A, Ponia SS, Tripathi S, Balasubramaniam V, Miorin L, Sourisseau M et al (2016) Zika virus targets human STAT2 to inhibit type I interferon signaling. Cell Host Microbe 19(6):882–890. https://doi.org/10.1016/j.chom.2016.05.009

Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, Luplertlop N et al (2015) Biology of Zika virus infection in human skin cells. J Virol 89(17):8880–8896. https://doi.org/10.1128/jvi.00354-15

Han YW, Choi JY, Uyangaa E, Kim SB, Kim JH, Kim BS et al (2014) Distinct dictation of Japanese encephalitis virus-induced neuroinflammation and lethality via triggering TLR3 and TLR4 signal pathways. PLoS Pathog 10(9):e1004319. https://doi.org/10.1371/journal.ppat.1004319

Hastings AK, Hastings K, Uraki R, Hwang J, Gaitsch H, Dhaliwal K et al (2019) Loss of the TAM receptor Axl ameliorates severe Zika virus pathogenesis and reduces apoptosis in microglia. iScience 13:339–350. https://doi.org/10.1016/j.isci.2019.03.003

Hategan A, Bianchet MA, Steiner J, Karnaukhova E, Masliah E, Fields A et al (2017) HIV tat protein and amyloid-β peptide form multifibrillar structures that cause neurotoxicity. Nat Struct Mol Biol 24(4):379–386. https://doi.org/10.1038/nsmb.3379

Hazleton JE, Berman JW, Eugenin EA (2012) Purinergic receptors are required for HIV-1 infection of primary human macrophages. J Immunol 188(9):4488–4495. https://doi.org/10.4049/jimmunol.1102482

He Z, Chen J, Zhu X, An S, Dong X, Yu J et al (2018) NLRP3 inflammasome activation mediates zika virus–associated inflammation. J Infect Dis 217(12):1942–1951

He Z, An S, Chen J, Zhang S, Tan C, Yu J et al (2020) Neural progenitor cell pyroptosis contributes to Zika virus-induced brain atrophy and represents a therapeutic target. Proc Natl Acad Sci U S A 117(38):23869–23878. https://doi.org/10.1073/pnas.2007773117

Herz J, Johnson KR, McGavern DB (2015) Therapeutic antiviral T cells noncytopathically clear persistently infected microglia after conversion into antigen-presenting cells. J Exp Med 212(8): 1153–1169. https://doi.org/10.1084/jem.20142047

Honda K, Ohba Y, Yanai H, Negishi H, Mizutani T, Takaoka A et al (2005) Spatiotemporal regulation of MyD88–IRF-7 signalling for robust type-I interferon induction. Nature 434(7036): 1035–1040. https://doi.org/10.1038/nature03547

Huang Z, Wu S-Q, Liang Y, Zhou X, Chen W, Li L et al (2015) RIP1/RIP3 binding to HSV-1 ICP6 initiates necroptosis to restrict virus propagation in mice. Cell Host Microbe 17(2):229–242

Huang Y, Wang Y, Meng S, Chen Z, Kong H, Pan T et al (2020) Autophagy contributes to host immunity and protection against Zika virus infection via type I IFN signaling. Mediat Inflamm 2020:9527147. https://doi.org/10.1155/2020/9527147

Hüfner K, Derfuss T, Herberger S, Sunami K, Russell S, Sinicina I et al (2006) Latency of alpha-herpes viruses is accompanied by a chronic inflammation in human trigeminal ganglia but not in dorsal root ganglia. J Neuropathol Exp Neurol 65(10):1022–1030. https://doi.org/10.1097/01.jnen.0000235852.92963.bf

Ichiyama T, Ito Y, Kubota M, Yamazaki T, Nakamura K, Furukawa S (2009) Serum and cerebrospinal fluid levels of cytokines in acute encephalopathy associated with human herpesvirus-6 infection. Brain and Development 31(10):731–738. https://doi.org/10.1016/j.braindev.2008.11.005

Jhan MK, Tsai TT, Chen CL, Tsai CC, Cheng YL, Lee YC et al (2017) Dengue virus infection increases microglial cell migration. Sci Rep 7(1):91. https://doi.org/10.1038/s41598-017-00182-z

Kallfass C, Ackerman A, Lienenklaus S, Weiss S, Heimrich B, Staeheli P (2012) Visualizing production of beta interferon by astrocytes and microglia in brain of La Crosse virus-infected mice. J Virol 86(20):11223–11230. https://doi.org/10.1128/jvi.01093-12

Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K et al (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441(7089):101–105. https://doi.org/10.1038/nature04734

Käufer C, Chhatbar C, Bröer S, Waltl I, Ghita L, Gerhauser I et al (2018) Chemokine receptors CCR2 and CX3CR1 regulate viral encephalitis-induced hippocampal damage but not seizures. Proc Natl Acad Sci U S A 115(38):E8929–E8938. https://doi.org/10.1073/pnas.1806754115%J

Kaushik DK, Gupta M, Kumawat KL, Basu A (2012) NLRP3 inflammasome: key mediator of neuroinflammation in murine Japanese encephalitis. PLoS One 7(2):e32270. https://doi.org/10.1371/journal.pone.0032270

Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat Immunol 11(5):373–384. https://doi.org/10.1038/ni.1863

Kawai T, Sato S, Ishii KJ, Coban C, Hemmi H, Yamamoto M et al (2004) Interferon-alpha induction through toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. Nat Immunol 5(10):1061–1068. https://doi.org/10.1038/ni1118

Keck F, Kortchak S, Bakovic A, Roberts B, Agrawal N, Narayanan A (2018) Direct and indirect pro-inflammatory cytokine response resulting from TC-83 infection of glial cells. Virulence 9(1):1403–1421. https://doi.org/10.1080/21505594.2018.1509668

Khanna KM, Lepisto AJ, Decman V, Hendricks RL (2004) Immune control of herpes simplex virus during latency. Curr Opin Immunol 16(4):463–469. https://doi.org/10.1016/j.coi.2004.05.003

Killian MS (2012) Dual role of autophagy in HIV-1 replication and pathogenesis. AIDS Res Ther 9(1):16. https://doi.org/10.1186/1742-6405-9-16

Kumar DK, Choi SH, Washicosky KJ, Eimer WA, Tucker S, Ghofrani J et al (2016) Amyloid-β peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. Sci Transl Med 8(340):340ra372. https://doi.org/10.1126/scitranslmed.aaf1059

Kurt-Jones EA, Chan M, Zhou S, Wang J, Reed G, Bronson R et al (2004) Herpes simplex virus 1 interaction with toll-like receptor 2 contributes to lethal encephalitis. Proc Natl Acad Sci U S A 101(5):1315–1320. https://doi.org/10.1073/pnas.0308057100

Kvansakul M (2017) Viral infection and apoptosis. Viruses 9(12). https://doi.org/10.3390/v9120356

Lavi E, Cong L (2020) Type I astrocytes and microglia induce a cytokine response in an encephalitic murine coronavirus infection. Exp Mol Pathol 115:104474. https://doi.org/10.1016/j.yexmp.2020.104474

Ledur PF, Karmirian K, da Silva Gouveia Pedrosa C, Souza LRQ, Assis-de-Lemos G, Martins TM et al (2020) Zika virus infection leads to mitochondrial failure, oxidative stress and DNA

damage in human iPSC-derived astrocytes. Sci Rep 10(1):1218. https://doi.org/10.1038/s41598-020-57914-x

Lee H-C, Chathuranga K, Lee J-S (2019) Intracellular sensing of viral genomes and viral evasion. Exp Mol Med 51(12):1–13. https://doi.org/10.1038/s12276-019-0299-y

Lellouch-Tubiana A, Fohlen M, Robain O, Rozenberg F (2000) Immunocytochemical characterization of long-term persistent immune activation in human brain after herpes simplex encephalitis. Neuropathol Appl Neurobiol 26(3):285–294. https://doi.org/10.1046/j.1365-2990.2000.00243.x

Lim, Hye Kyung, Seppänen, Mikko, Hautala, Timo, Ciancanelli, Michael J., Itan, Yuval, Lafaille, Fabien G., . . . Zhang, Shen-Ying. (2014). TLR3 deficiency in herpes simplex encephalitis: high allelic heterogeneity and recurrence risk. Neurology, 83(21), 1888–1897. doi:https://doi.org/10.1212/WNL.0000000000000999

Liu Y, Gordesky-Gold B, Leney-Greene M, Weinbren NL, Tudor M, Cherry S (2018) Inflammation-induced, STING-dependent autophagy restricts Zika virus infection in the drosophila brain. Cell Host Microbe 24(1):57–68.e53. https://doi.org/10.1016/j.chom.2018.05.022

Ludlow M, Kortekaas J, Herden C, Hoffmann B, Tappe D, Trebst C et al (2016) Neurotropic virus infections as the cause of immediate and delayed neuropathology. Acta Neuropathol 131(2):159–184. https://doi.org/10.1007/s00401-015-1511-3

Madhu BP, Singh KP, Saminathan M, Singh R, Shivasharanappa N, Sharma AK et al (2016) Role of nitric oxide in the regulation of immune responses during rabies virus infection in mice. Virusdisease 27(4):387–399. https://doi.org/10.1007/s13337-016-0343-7

Malone KE, Stohlman SA, Ramakrishna C, Macklin W, Bergmann CC (2008) Induction of class I antigen processing components in oligodendroglia and microglia during viral encephalomyelitis. Glia 56(4):426–435. https://doi.org/10.1002/glia.20625

Mateo R, Nagamine CM, Spagnolo J, Méndez E, Rahe M, Gale M Jr et al (2013) Inhibition of cellular autophagy deranges dengue virion maturation. J Virol 87(3):1312–1321. https://doi.org/10.1128/jvi.02177-12

Menachery VD, Pasieka TJ, Leib DA (2010) Interferon regulatory factor 3-dependent pathways are critical for control of herpes simplex virus type 1 central nervous system infection. J Virol 84(19):9685–9694. https://doi.org/10.1128/jvi.00706-10

Mishra MK, Ghosh D, Duseja R, Basu A (2009) Antioxidant potential of minocycline in Japanese encephalitis virus infection in murine neuroblastoma cells: correlation with membrane fluidity and cell death. Neurochem Int 54(7):464–470. https://doi.org/10.1016/j.neuint.2009.01.022

Mishra R, Chhatbar C, Singh SK (2012) HIV-1 Tat C-mediated regulation of tumor necrosis factor receptor-associated factor-3 by microRNA 32 in human microglia. J Neuroinflammation 9(1):131. https://doi.org/10.1186/1742-2094-9-131

Moseman EA, Blanchard AC, Nayak D, McGavern DB (2020) T cell engagement of cross-presenting microglia protects the brain from a nasal virus infection. J Sci Immunol 5(48):eabb1817. https://doi.org/10.1126/sciimmunol.abb1817

Nazmi A, Mukherjee S, Kundu K, Dutta K, Mahadevan A, Shankar SK, Basu A (2014) TLR7 is a key regulator of innate immunity against Japanese encephalitis virus infection. Neurobiol Dis 69:235–247. https://doi.org/10.1016/j.nbd.2014.05.036

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318. https://doi.org/10.1126/science.1110647

Nunes P, Nogueira R, Coelho J, Rodrigues F, Salomão N, José C et al (2019) A stillborn multiple organs' investigation from a maternal DENV-4 infection: histopathological and inflammatory mediators characterization. Viruses 11(4):319

O'Connell D, Liang C (2016) Autophagy interaction with herpes simplex virus type-1 infection. Autophagy 12(3):451–459. https://doi.org/10.1080/15548627.2016.1139262

ten Oever, Benjamin R., Ng, Sze-Ling, Chua, Mark A., McWhirter, Sarah M., García-Sastre, Adolfo, & Maniatis, Tom (2007). Multiple functions of the IKK-related kinase IKKε in

interferon-mediated antiviral immunity. 315(5816), 1274–1278. doi:https://doi.org/10.1126/science.1136567 %J Science

Olson JK, Girvin AM, Miller SD (2001) Direct activation of innate and antigen-presenting functions of microglia following infection with Theiler's virus. J Virol 75(20):9780–9789. https://doi.org/10.1128/JVI.75.20.9780-9789.2001

Ortega M, Ances BM (2014) Role of HIV in amyloid metabolism. J Neuroimmune Pharmacol 9(4): 483–491. https://doi.org/10.1007/s11481-014-9546-0

Orvedahl A, MacPherson S, Sumpter R Jr, Tallóczy Z, Zou Z, Levine B (2010) Autophagy protects against Sindbis virus infection of the central nervous system. Cell Host Microbe 7(2):115–127. https://doi.org/10.1016/j.chom.2010.01.007

Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T (2003) TICAM-1, an adaptor molecule that participates in Toll-like receptor 3–mediated interferon-β induction. Nat Immunol 4(2): 161–167. https://doi.org/10.1038/ni886

Pagani F, Paolicelli RC, Murana E, Cortese B, Di Angelantonio S, Zurolo E et al (2015) Defective microglial development in the hippocampus of Cx3cr1 deficient mice. Front Cell Neurosci 9: 111. https://doi.org/10.3389/fncel.2015.00111

Perfettini JL, Castedo M, Roumier T, Andreau K, Nardacci R, Piacentini M, Kroemer G (2005) Mechanisms of apoptosis induction by the HIV-1 envelope. Cell Death Differ 12(Suppl 1): 916–923. https://doi.org/10.1038/sj.cdd.4401584

Periyasamy P, Thangaraj A, Guo ML, Hu G, Callen S, Buch S (2018) Epigenetic promoter DNA methylation of miR-124 promotes HIV-1 tat-mediated microglial activation via MECP2-STAT3 axis. J Neurosci 38(23):5367–5383. https://doi.org/10.1523/jneurosci.3474-17.2018

Periyasamy P, Thangaraj A, Bendi VS, Buch S (2019) HIV-1 Tat-mediated microglial inflammation involves a novel miRNA-34a-NLRC5-NFκB signaling axis. Brain Behav Immun 80:227–237. https://doi.org/10.1016/j.bbi.2019.03.011

Persidsky Y, Stins M, Way D, Witte MH, Weinand M, Kim KS et al (1997) A model for monocyte migration through the blood-brain barrier during HIV-1 encephalitis. J Immunol 158(7): 3499–3510

Platanias LC (2005) Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol 5(5):375–386. https://doi.org/10.1038/nri1604

Priya R, Patro IK, Parida MM (2014) TLR3 mediated innate immune response in mice brain following infection with chikungunya virus. Virus Res 189:194–205. https://doi.org/10.1016/j.virusres.2014.05.010

Quick ED, Leser JS, Clarke P, Tyler KL (2014) Activation of intrinsic immune responses and microglial phagocytosis in an ex vivo spinal cord slice culture model of West Nile virus infection. J Virol 88(22):13005–13014. https://doi.org/10.1128/jvi.01994-14

Rastogi M, Singh SK (2020a) Japanese encephalitis virus exploits microRNA-155 to suppress the non-canonical NF-κB pathway in human microglial cells. Biochim Biophys Acta Gene Regul Mech 1863(11):194639. https://doi.org/10.1016/j.bbagrm.2020.194639

Rastogi M, Singh SK (2020b) Zika virus NS1 affects the junctional integrity of human brain microvascular endothelial cells. Biochimie 176:52–61. https://doi.org/10.1016/j.biochi.2020.06.011

Ravi V, Desai AS, Shenoy PK, Satishchandra P, Chandramuki A, Gourie-Devi M (1993) Persistence of Japanese encephalitis virus in the human nervous system. J Med Virol 40(4):326–329. https://doi.org/10.1002/jmv.1890400412

Reinert LS, Lopušná K, Winther H, Sun C, Thomsen MK, Nandakumar R et al (2016) Sensing of HSV-1 by the cGAS–STING pathway in microglia orchestrates antiviral defence in the CNS. Nat Commun 7(1):13348. https://doi.org/10.1038/ncomms13348

Reinert LS, Rashidi AS, Tran DN, Katzilieris-Petras G, Hvidt AK, Gohr M et al (2021) Brain immune cells undergo cGAS/STING-dependent apoptosis during herpes simplex virus type 1 infection to limit type I IFN production. J Clin Invest 131(1). https://doi.org/10.1172/jci136824

Rodríguez-Gómez JA, Kavanagh E, Engskog-Vlachos P, Engskog MKR, Herrera AJ, Espinosa-Oliva AM et al (2020) Microglia: agents of the CNS pro-inflammatory response. Cells 9(7): 1717

Ronca SE, Dineley KT, Paessler S (2016) Neurological sequelae resulting from encephalitic alphavirus infection. Front Microbiol 7:959–959. https://doi.org/10.3389/fmicb.2016.00959

Rosati A, Khalili K, Deshmane SL, Radhakrishnan S, Pascale M, Turco MC, Marzullo L (2009) BAG3 protein regulates caspase-3 activation in HIV-1-infected human primary microglial cells. J Cell Physiol 218(2):264–267. https://doi.org/10.1002/jcp.21604

Schreiber G, Piehler J (2015) The molecular basis for functional plasticity in type I interferon signaling. Trends Immunol 36(3):139–149. https://doi.org/10.1016/j.it.2015.01.002

Séror C, Melki MT, Subra F, Raza SQ, Bras M, Saïdi H et al (2011) Extracellular ATP acts on P2Y2 purinergic receptors to facilitate HIV-1 infection. J Exp Med 208(9):1823–1834. https://doi.org/10.1084/jem.20101805

Sharma N, Verma R, Kumawat KL, Basu A, Singh SK (2015) miR-146a suppresses cellular immune response during Japanese encephalitis virus JaOArS982 strain infection in human microglial cells. J Neuroinflammation 12(1):30. https://doi.org/10.1186/s12974-015-0249-0

Shigemoto-Mogami Y, Hoshikawa K, Sato K (2018) Activated microglia disrupt the blood-brain barrier and induce chemokines and cytokines in a rat in vitro. Model 12(494). https://doi.org/10.3389/fncel.2018.00494

Shrestha B, Zhang B, Purtha WE, Klein RS, Diamond MS (2008) Tumor necrosis factor alpha protects against lethal West Nile virus infection by promoting trafficking of mononuclear leukocytes into the central nervous system. J Virol 82(18):8956–8964. https://doi.org/10.1128/jvi.01118-08

Simões JLB, Bagatini MD (2021) Purinergic signaling of ATP in COVID-19 associated Guillain-Barré syndrome. J Neuroimmune Pharmacol 16(1):48–58. https://doi.org/10.1007/s11481-020-09980-1

Singh S, Singh G, Tiwari S, Kumar A (2020) CCR2 inhibition reduces neurotoxic microglia activation phenotype after Japanese encephalitis viral. Infection 14(230). https://doi.org/10.3389/fncel.2020.00230

Singh H, Koury J, Kaul M (2021) Innate immune sensing of viruses and its consequences for the central nervous system. Viruses 13(2):170

Solanki A, Radotra BD, Vasishta RK (2009) Correlation of cytokine expression with rabies virus distribution in rabies encephalitis. J Neuroimmunol 217(1):85–89. https://doi.org/10.1016/j.jneuroim.2009.09.019

Sørensen LN, Reinert LS, Malmgaard L, Bartholdy C, Thomsen AR, Paludan SR (2008) TLR2 and TLR9 synergistically control herpes simplex virus infection in the brain. J Immunol 181(12): 8604–8612. https://doi.org/10.4049/jimmunol.181.12.8604%J

Sperlágh B, Illes P (2007) Purinergic modulation of microglial cell activation. Purinergic Signal 3(1–2):117–127. https://doi.org/10.1007/s11302-006-9043-x

Sun L, Wu J, Du F, Chen X, Chen ZJ (2013) Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339(6121):786–791. https://doi.org/10.1126/science.1232458

Sutherland EJ, Brew BJ (2018) Human immunodeficiency virus and the nervous system. Neurol Clin 36(4):751–765. https://doi.org/10.1016/j.ncl.2018.07.002

Tan IL, Smith BR, von Geldern G, Mateen FJ, McArthur JC (2012) HIV-associated opportunistic infections of the CNS. Lancet Neurol 11(7):605–617. https://doi.org/10.1016/s1474-4422(12)70098-4

Theil D, Derfuss T, Paripovic I, Herberger S, Meinl E, Schueler O et al (2003) Latent herpesvirus infection in human trigeminal ganglia causes chronic immune response. Am J Pathol 163(6): 2179–2184. https://doi.org/10.1016/S0002-9440(10)63575-4

Thongtan T, Cheepsunthorn P, Chaiworakul V, Rattanarungsan C, Wikan N, Smith DR (2010) Highly permissive infection of microglial cells by Japanese encephalitis virus: a possible role as a viral reservoir. Microbes Infect 12(1):37–45. https://doi.org/10.1016/j.micinf.2009.09.013

Town T, Jeng D, Alexopoulou L, Tan J, Flavell RA (2006) Microglia recognize double-stranded RNA via TLR3. J Immunol 176(6):3804–3812. https://doi.org/10.4049/jimmunol.176.6.3804%J

Town T, Bai F, Wang T, Kaplan AT, Qian F, Montgomery RR et al (2009) Toll-like receptor 7 mitigates lethal West Nile encephalitis via interleukin 23-dependent immune cell infiltration and homing. Immunity 30(2):242–253. https://doi.org/10.1016/j.immuni.2008.11.012

Tricarico PM, Caracciolo I, Crovella S, D'Agaro P (2017) Zika virus induces inflammasome activation in the glial cell line U87-MG. Biochem Biophys Res Commun 492(4):597–602. https://doi.org/10.1016/j.bbrc.2017.01.158

Tsai T-T, Chen C-L, Lin Y-S, Chang C-P, Tsai C-C, Cheng Y-L et al (2016) Microglia retard dengue virus-induced acute viral encephalitis. Sci Rep 6(1):27670. https://doi.org/10.1038/srep27670

Turpin J, Frumence E, Desprès P, Viranaicken W, Krejbich-Trotot P (2019) The ZIKA virus delays cell death through the anti-apoptotic Bcl-2 family proteins. Cell 8(11). https://doi.org/10.3390/cells8111338

Vasek, Michael J., Garber, Charise, Dorsey, Denise, Durrant, Douglas M., Bollman, Bryan, Soung, Allison, . . . Klein, Robyn S. (2016). A complement–microglial axis drives synapse loss during virus-induced memory impairment. Nature, 534(7608), 538–543. doi:https://doi.org/10.1038/nature18283

Verma R, Bharti K (2017) Toll like receptor 3 and viral infections of nervous system. J Neurol Sci 372:40–48. https://doi.org/10.1016/j.jns.2016.11.034

Verma AK, Ghosh S, Pradhan S, Basu A (2016) Microglial activation induces neuronal death in Chandipura virus infection. Sci Rep 6(1):22544. https://doi.org/10.1038/srep22544

Vuaillat C, Varrin-Doyer M, Bernard A, Sagardoy I, Cavagna S, Chounlamountri I et al (2008) High CRMP2 expression in peripheral T lymphocytes is associated with recruitment to the brain during virus-induced neuroinflammation. J Neuroimmunol 193(1–2):38–51. https://doi.org/10.1016/j.jneuroim.2007.09.033

Wallet C, De Rovere M, Van Assche J, Daouad F, De Wit S, Gautier V et al (2019) Microglial cells: the main HIV-1 reservoir in the brain. Front Cell Infect Microbiol 9(362). https://doi.org/10.3389/fcimb.2019.00362

Waltl I, Käufer C, Gerhauser I, Chhatbar C, Ghita L, Kalinke U, Löscher W (2018) Microglia have a protective role in viral encephalitis-induced seizure development and hippocampal damage. Brain Behav Immun 74:186–204. https://doi.org/10.1016/j.bbi.2018.09.006

Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA (2004) Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat Med 10(12):1366–1373. https://doi.org/10.1038/nm1140

Wheeler DL, Sariol A, Meyerholz DK, Perlman S (2018) Microglia are required for protection against lethal coronavirus encephalitis in mice. J Clin Invest 128(3):931–943. https://doi.org/10.1172/jci97229

Williams DW, Byrd D, Rubin LH, Anastos K, Morgello S, Berman JW (2014) CCR2 on CD14+CD16+ monocytes is a biomarker of HIV-associated neurocognitive disorders. Neurol Neuroimmunol Neuroinflamm 1(3):e36. https://doi.org/10.1212/NXI.0000000000000036%J

Wong ME, Jaworowski A, Hearps AC (2019) Corrigendum: the HIV reservoir in monocytes and macrophages. Front Immunol 10:2517. https://doi.org/10.3389/fimmu.2019.02517

Xu P, Shan C, Dunn TJ, Xie X, Xia H, Gao J et al (2021) Correction: role of microglia in the dissemination of Zika virus from mother to fetal brain. PLoS Negl Trop Dis 15(4):e0009344. https://doi.org/10.1371/journal.pntd.0009344

Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H et al (2003) Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 301(5633):640–643. https://doi.org/10.1126/science.1087262

Zhao P, Yang Y, Feng H, Zhao L, Qin J, Zhang T et al (2013) Global gene expression changes in BV2 microglial cell line during rabies virus infection. Infect Genet Evol 20:257–269. https://doi.org/10.1016/j.meegid.2013.09.016

Zhou Y, Danbolt NC (2014) Glutamate as a neurotransmitter in the healthy brain. J Neural Transm 121(8):799–817. https://doi.org/10.1007/s00702-014-1180-8

Zimmermann J, Hafezi W, Dockhorn A, Lorentzen EU, Krauthausen M, Getts DR et al (2017) Enhanced viral clearance and reduced leukocyte infiltration in experimental herpes encephalitis after intranasal infection of CXCR3-deficient mice. J Neurovirol 23(3):394–403. https://doi.org/10.1007/s13365-016-0508-6

Microglia Aging

Nisha Patro, Sarika Singh Kushwaha, and Ishan Patro

Abstract

"Microglia aging" is about the aging of microglia and its role in the aging brain. With age, microglia get converted to divergent phenotypes with diverse functional responsibilities. Microglia are immunocompetent cells of the nervous system, constitute about 10% of the total glial cells, and are the first to sense any damage due to infectious, traumatic, inflammatory, ischemic, and degenerative disease of the CNS. Microglia are generated early in life, live long, and are active through their life. The age of an individual reflects the aging changes in microglia. Very little is known about the changes that occur in the microglia with normal aging. With age they get primed and respond to such processes differently. Microglia are primarily responsible for removal of the debris, and with age, debris starts to accumulate in the form of lipofuscin in themselves. Counteracting aging in microglia is obviously a new challenge for neurobiologists. Eliminating aged or senescent microglia and repopulating with new microglia in aging and neurodegenerative brain help to attain microglial cell densities similar to young adult animals and improve cognition. This chapter intends to discuss the issues in detail.

Keywords

Aging · Brain aging · Microglial heterogeneity · Aging of microglia · Modelling aging microglia

N. Patro · S. S. Kushwaha · I. Patro (✉)
School of Studies in Neuroscience, Jiwaji University, Gwalior, India

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_20

1 Introduction

"Microglia aging" is about the aging of microglia and the role of microglia in the aging brain. With age, microglia get converted to divergent phenotypes with diverse functional responsibilities. Microglia are immunocompetent cells of the nervous system, constitute about 10% of the brain cells, and are the first to sense any damage due to infectious, traumatic, inflammatory, ischemic, and degenerative disease process of the central nervous system (CNS). Microglia are generated early in life, live long, and are active through their life. Age of an individual reflects the aging changes in microglia. Very little is known about the changes that occur in microglia with normal aging. With age, they get primed and respond to such processes differently. Microglia are primarily responsible for removal of the debris, and with age, debris starts to accumulate in the form of lipofuscin in themselves. Counteracting aging in microglia is obviously a new challenge for neurobiologists. Eliminating aged or senescent microglia and repopulating with new microglia in aging and neurodegenerative brain help to attain microglial cell densities similar to young adult animals and improve cognition. This chapter intends to discuss the issues in detail.

Aging continuously accumulates changes with time and life of an individual that brings in functional changes to the brain (as also other tissues) leading to an agglomeration of cells that have lost their ability to divide and have accrued a pro-inflammatory phenotype that negatively impacts on its microenvironment and finally on the functional physiology of an individual. Hayflick and Moorhead (1961) first proposed the concept of cellular aging and explained a dysfunctional and growth arrested state as senescence. Such changes along with others collectively bring about malignancies, immobility, loss of cognition, and frailty (reviewed by Swenson et al. 2019). These senescent cells, both neurons and glia, have been associated with neurodegenerative disorders where age plays a major role. With an increase in longevity of man, neurodegenerative disorders have become a prime health concern.

The past two decades of research has frequently evidenced the role of microglia in neurodegenerative disorders. The supportive and protective role of microglia has also been emphasized. The logical interpretation could be a part of the microglial population is responsible for this. It is also understood that most neurodegenerative diseases manifest past 50 years of age. Thus, it could be an acceptable proposition that the senescent/dystrophic microglia which are quantitatively high in aging brain are evidently involved in neurodegeneration. Thus, the role of aging of microglia and role of microglia in the aging brain are both equally important to study.

Microglia are the immunocompetent cells of the nervous system and represent about 10% of all glial cells in the brain (Town et al. 2005) and are the first to sense any damage due to infectious, traumatic, inflammatory, ischemic, and degenerative disease processes in the CNS (Santambrogio et al. 2001; Patro et al. 2005; Tambuyzer et al. 2009; Patro et al. 2010, 2016; Sharma et al. 2016). Once stimulated, they undergo profound immunophenotypic and functional changes, migrate to the injured site, proliferate and phagocytose the cell debris and apoptotic cells, release cytokines to maintain the homeostasis, and support injured neurons,

hence helping in neuronal survival (Stoll and Jander 1999; Raivich et al. 1999; Saxena et al. 2007; Tambuyzer et al. 2009). In contrast, chronic low-level inflammation which is common in the aging brain has been identified as a major contributor to most neurodegenerative diseases. Alterations in the aged brain induce microgliosis leading to the production of pro-inflammatory and neurotoxic mediators that induce neurodegeneration and further activation of microglia (Miller and Streit 2007).

However, we also have evidence that microglia themselves are subject to age-related structural deterioration and replicative senescence, diminution of neuroprotective functions and dysregulated responses to signals, and alterations in their environment (Flanary and Streit 2004; Streit et al. 2004, 2008; Streit 2006; Luo et al. 2010). The senescent microglia in aged brain become pro-inflammatory and secrete various cytokines, chemokines, matrix metalloproteases, etc. capable of disarranging the functional chains of the neuronal circuitries. When these microglia are exposed even further, they contribute to ageing associated patho-physiological alterations as also cognitive impairments. Such microglial senescence is reported to be exacerbated in the presence of amyloid-β (Aβ), suggesting that Aβ adversely affects the physiological functions of microglia by hastening their structural decline (Korotzer et al. 1993).

While the brain is in a state of normal homeostasis, the microglia continue to survey their microenvironment. They are then seen as resting state designated as M_0, ramified with small cell body and motile processes (Tremblay et al. 2010). Aging can negatively influence both resident and infiltrating macrophages with respect to population of cells as well as their secreting profiles and more toward a pro-inflammatory state (Xie et al. 2003; Godbout et al. 2005; Swenson et al. 2019). Aging also impairs microglia's phagocytic proficiency. Microglia in the senile brain tend to be converted to M_2 neuroprotective phenotype (Hickman et al. 2013). The chronically activated M_1 aged microglia on the other hand restrict the neuroblasts to the site of damage to establish new neural connections (Moraga et al. 2015). Such pro-inflammatory signals when sensed by the normal cells in the brain may influence them to have impaired response to injury or insults, and the cells may even dysfunction.

Recently, Elmore and group (2018) repopulated senile mice brain with microglia from young mice to observe something quite challenging for glia biologists to explore. Such repopulation effectively reversed the gene expression anomalies like those for cytoskeletal remodeling and synaptogenesis that occur with aging. Changes in cellular organization and deformities were reversed both by elimination of senile microglia and repopulation by younger healthy microglia. Microglial elimination in the senile brain encouraged adult neurogenesis and dendritic spine densities. Along with cellular health, the mice repopulated with younger microglia presented better long-term potentiation that was lost to age.

2 Microglial Aging and Brain Health

Our understanding of age-associated change in microglia, its turnover, morphology, and phenotypes is limited. Aging could bring in cellular changes in the microglia resulting in increased disability in these cells in providing neuroprotection following injury and/or stress in the elderly. The age-affected microglia were described as senescent or dystrophic by Streit et al. (2004), and whether these two states are different remains to be established. Such activated microglia remain physiologically active and secrete a completely different set of molecules including cytokines and lose the ability to protect neurons from challenges (insults) and are of 'senescence-associated secretory phenotype' (SASP) (Chinta et al. 2015).

Senescent or dystrophic microglia are believed to cause decline in neuronal function in the aging brain, but involvement of such microglia in the demyelinated diseases remains to be explored (Angelova and Brown 2019). Aged or senescent microglia are believed to induce impairment of neuronal activity in senile brains although there is no concrete evidence to believe this. Bussian et al. (2018) induced suicide protein in senescent microglia in a tau-based neurodegeneration model that helped in restricting the pathology indicating the pathophysiological implications of such microglia.

Aberrant inflammatory responses mediated by the microglia play a vital role in the pathogenesis of many age-related neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and macular degeneration (Palace 2007; Xu et al. 2009; Perry et al. 2010) where the coexistence of neuroinflammation and neurodegeneration has been demonstrated (Klegeris et al. 2007; Esiri 2007). The presence of degenerating microglial cells in the aged human brain as well as in the brains of the humans and animals with neurodegenerative diseases has been widely demonstrated (Conde and Streit 2006; Streit 2006; Fendrick et al. 2007). Based on the findings on such degenerating microglia, a microglial dysfunction hypothesis was put forward, stating that senescence of microglia produces dysfunctional cells, losing their neuroprotective abilities, justifying that the incidence of neurodegenerative diseases increases with age (Streit et al. 2008). However, all these data are sporadic and known from studies addressing factors other than age as a primary cause. It still remains to understand how aging affects microglial function. In view of the neuroprotective effects of microglia, certain studies have reported a direct correlation between advancing age and increased risk of poor recovery from traumatic brain injury (Hukkelhoven et al. 2003).

3 Aging of Microglia

Microglia have a different origin than the macroglia (astrocytes and oligodendroglia). We have several debates on the exact origin of microglia. Now we agree to the proposition that microglia are derived from yolk sac macrophage precursors (Prinz and Priller 2014). More details on the origin and maturation of macroglia and microglia have been discussed in detail in two of the earlier chapters in this book.

Microglia play important role in development process like neurogenesis, synaptic pruning, and neuronal networks (Prinz and Priller 2014). As microglia are not continuously renewed, they are vulnerable to age and associated physiological state and thus contribute to the onset of neurodegenerative diseases and their progression and to the functional damage that follows.

However, very little is known about the changes that occur in microglia with normal aging. Among the known are; immunophenotypic changes in microglia, upregulated expression of MHC class II molecules, pro-inflammatory cytokines (TNFα, IL-1β, IL-6, and IL-12b/p40 mRNAs) and decreased anti-inflammatory cytokines (TGFβ and IL-10 mRNAs), increased ED1 macrophage marker immunoreactivity, and phagocytic morphology (Perry et al. 1993; Ogura et al. 1994; Sheng et al. 1997; Sheffield and Berman 1998; Ye and Johnson 1999; Kullberg et al. 2001; Godbout and Johnson 2006; Sierra et al. 2007; Stichel and Luebbert 2007). All the above evidences suggest that the microglia become primed and over-responsive to even overt stimuli and show uncontrolled activation that can contribute to neurodegeneration (Luo et al. 2010). Several morphological abnormalities in microglial cytoplasmic structure leading to microglial dystrophy developing as a result of microglial senescence have also been reported in the aging human brain (Streit et al. 2004; Wasserman et al. 2008). Such dystrophic changes are clearly different from morphological changes that occur during microglial activation observed in rodents following acute CNS injury. Microglial senescence has also been related to significant telomere shortening and reduction of telomerase activity that occurs during normal aging in rats. We need to understand how age as the sole parameter induces microglial senescence, leading to the loss of their multifunctional ability, and as to how the microglia respond to the normal process of aging, in the aging brain, and as it ages by itself.

It is now understood that the microglia never rest. They are always in a state of surveillance under the influence of factors released by healthy neurons (Conrad and Dittel 2011). Such microglia are of several morphological states with constantly extending and protruding processes that keep contacting the neuronal synapses and chemically regulate them (Hristovska and Pascual 2015) until they identify any potential threat to the neurons to turn active – this is the immune responsibility of microglia.

The senescent microglia usually are not uniformly distributed and have increased soma size and volume and effectively retracted and thick processes (Wong 2013) with a reduced motility of the processes leading to a significant decline in surveillance due to reduced synaptic contacts (Hefendehl et al. 2014). This is referred to as microglial atrophy that includes abnormal cell shape, deramification, and fragmented processes (Streit et al. 2004, 2009). Like the "dark" neurons (Sharma et al. 1988; James et al. 1992), the microglia in the normal aging brain have been described to have electron-dense cytoplasm and neoplasm as well as changes in nuclear chromatin. They are highly granular and stain darkly in most histological preparations (Bisht et al. 2016). Such granular and packed cytoplasm could be explained as defects in lysosomal digestion and related accumulation of cellular indigestible material including the lipofuscin pigment (Nakanishi and Wu 2009; Kushwaha et al. 2018).

Aged microglia present hyper expression of TNF-alpha, IL-1beta, IL-6, and IL-8 (Sierra et al. 2007); DNA damage (Coppé et al. 2010; Von Bernhardi et al. 2015); telomere shortening in dystrophic microglia (Flanary and Streit 2004); and so on.

4 Aging and Microglial Heterogeneity

Morphological diversity is a prominent feature with microglia. Such heterogeneity is more elaborate and prominent during development, aging, upon malnutrition and diseases (Patro et al. 2010; Sarkar et al. 2019; Sinha et al. 2019, 2020; Masuda et al. 2020; Delage et al. 2021) and is also linked with their detailed anatomy, structure, metabolism, proteome, and even epigenetic profile. The surveillant microglia in healthy brain have long, thin, and constantly extending and protruding processes, actively surveying the neurons and their synapses, blood vessels, parenchymal space, and other neighboring cell types in the brain (Nimmerjahn et al. 2005; Hristovska and Pascual 2015). In response to an injury, insult, any change in neuronal environment, or infection, the processes of such cells become shortened and thicken up but get back to their original state once the inflammation subsides (Patro et al. 2008, 2010, 2013). But in the aging or senile brain along with such surveying microglia, other morphologically and physiologically different microglia are also seen (Patro et al. 2010). These cells have fewer, stouter, and less motile processes (Shaerzadeh et al. 2020), have variable shape and enlarged soma (Sierra et al. 2007), and are believed to have a reduced functional ability and limited neuroprotective character because of aging.

Morphologically and physiologically distinct set of microglia are present in the aging and senile brain (Fig. 20.1); the dystrophic (as described above) and the gnarled microglia with bulbous swelling and fragmented processes (Streit et al. 2004); the rod-shaped microglia in the hippocampus, frontal, and parietal cortex in senile and/or demented human samples (Bachstetter et al. 2017) or following nerve injury (Saxena et al. 2007) or bacterial infection (Sharma et al. 2016) providing trophic support, neuroprotective activity and promote neuronal survival (Bachstetter et al. 2017).

With any stimuli that indicate neuronal damage, the microglia get activated and migrate to the site of injury (Nimmerjahn et al. 2005; Saxena et al. 2007) and then change both their morphology and molecular signature. The various activated states of microglia can be characteristically distinguished based on their morphology. Ramified microglia have highly motile thin processes. Non-phagocytic but reactive microglia possess thick and branched processes and show upregulation of MHCI/II, pro-inflammatory cytokines, and reactive oxygen species (ROS). They then get converted to phagocytic microglia that are large, ameboid, inflammatory, and able to phagocytose (Stopper et al. 2018).

The earliest description of aging changes in microglia comes from Streit et al. (2004). Several studies, including our own (Patro et al. 2010), have confirmed the microglial morphological changes they reported. Of course, additional information has also been added over the years. We have studied the microglial aging in rat brain

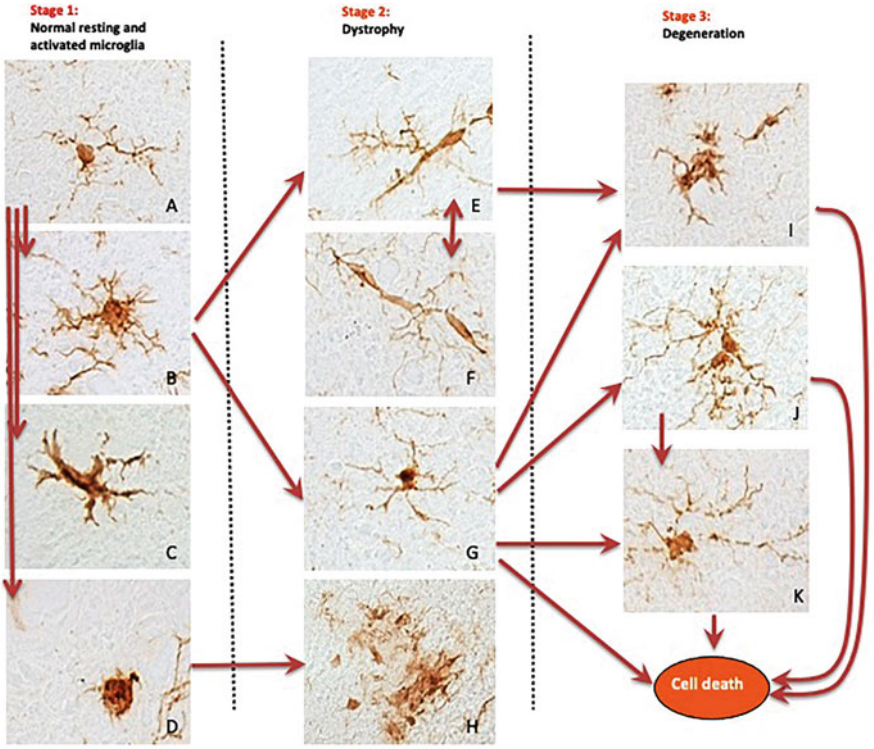

Fig. 20.1 Microglial phenotypes observed during normal aging. All the pictures are of similar magnification. (**a**) Normal resting microglia with small cell soma ramified, long and thin processes seen in 3-month-old rats; (**b**) hyper-ramified microglia with enlarged cell body and thick processes seen in 9–12-month-old rats; (**c** and **i**) atrophied microglia with lack of processes seen in 24-month-old and older rats; (**d**) phagocytic microglia with ameboid cell bodies seen in 24-month-old and older rats; (**e**) rod-shaped microglia with elongated cell body seen in 12-month-old and older rats; (**f**) end-to-end fused rod-shaped microglia seen in 12-month-old and older rats; (**g**) deramified microglia that have lost the finely branched processes seen in 18-month-old and older rats; (**h**) microglial clusters as seen at 24-month-old and older rats; (**j**) gnarled microglia with gnarled or twisted and stout processes seen in 30-month-old and older rats; and (**k**) beaded and fragmented microglia seen in 24-month-old and older rats

(hippocampus) across its life span, 21 days postpartum to 30 months of age. We have systematically explored (unpublished data) (1) the phenotypic and morphological changes in microglia, (2) density of microglial distribution, and (3) their activation profile in various subregions of the hippocampus across the life span of rats. A large number of microglia with thick hyper-ramified processes indicating an intermediate stage between resting and activated form were observed at 21 days of age, which could be due to the loss of neurons and their processes that occurs during maturation of the neuronal networking and related developmental events. At 3–6 months of age, the microglia were in resting stage with thin ramified processes and small cell body presenting typical ramified morphology. At several sites, microglial coupling was

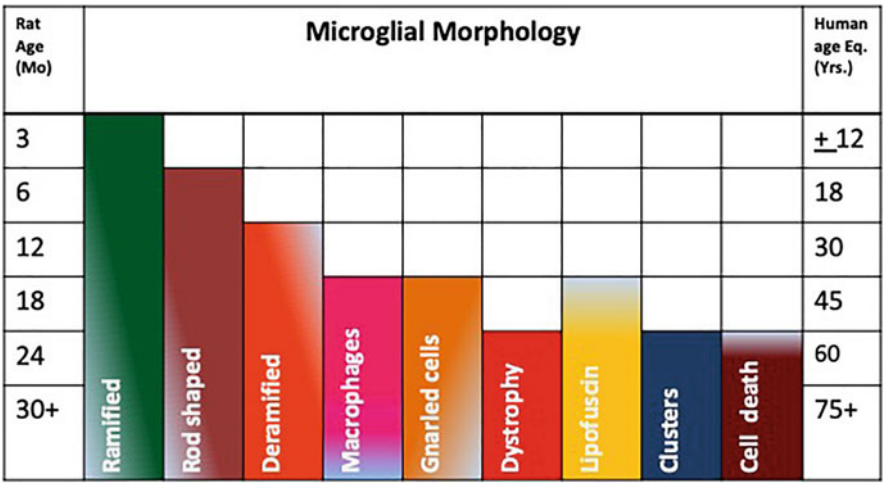

Fig. 20.2 Phenotypic timescale of microglia in the aging brain

observed with two cell bodies closely apposed to each other and joined to make a couple. By the age of 12 months, the microglia turned gradually reactive with thick and shortened processes and reduced ramification of their distal branches. Most microglia presented rod-shaped morphology with end-to-end fusion and coupling indicating the formal activated forms. The microglia became further activated by 18 months of age with hypertrophied and enlarged cell body with thick and short deramified processes and ameboid appearance. Microglia at this stage were MHC-II positive as well. Subsequently by the age of 24 months, most of the microglia presented various states of activation and dystrophy. The dystrophic microglia were seen with gnarled/twisted, deramified, and fragmented processes, giving beaded appearance with only some occasional normal microglia. Several of the microglia presented MHC II positivity as well. At this age point, the microglia began to fuse with each other, leading to the formation of small microglial clusters. At senility (30-month-old rats), the dystrophy of microglia was more pronounced, with very few normal ramified and activated microglia often coexisting side by side with dystrophic microglia possessing beaded, gnarled, deramified, and/or atrophied processes. A phenotypic timescale of microglia in rat brain (human equivalence as per Sengupta 2013) has been modeled in Fig. 20.2.

A gradual and progressive shift of the microglial morphology from normal to activated with increasing age has been reported. Thus, a stereological increase in microglial number with age and prominent phenotypic changes with upregulation of Iba1 in hippocampal subregions have been noted. Moreover by 18 months of age, most of the microglia remained in the activated primed states, expressing MHC II molecules and pro-inflammatory cytokines, viz., IL-1β, IL-6, and TNF-α. Although morphological plasticity is a characteristic feature of microglia, their primed status was maintained until senility and microglia subsequently become dystrophic. A

change in the ramified morphology of microglia in inflammation associated with numerous neurological disorders, such as AD and PD, has been variously reported to disrupt their intricate and essential synaptic functions leading to cognitive deficits (Paolicelli et al. 2011; Rogers et al. 2011; Morris et al. 2013). In neurodegenerative diseases, the microglia subsequent to a change in their phenotype can develop a pathological role, where they contribute to an increased rate of synaptic elimination compared to synaptogenesis as a possible mechanism to neurodegeneration and other pathologies (Walsh and Selkoe 2004; Morris et al. 2013).

When microglia are activated, they also undergo mitosis in regions of injury causing substantially increased microglial density (Graeber et al. 1988; Saxena et al. 2007; Patro et al. 2008).

5 Neuroinflammation in the Aging Brain

Microglial activation and neuroinflammation disturbs the homeostasis following injury. The microglia come to the rescue of the injured neurons, secrete neuroprotective cytokines, and render synaptic stripping, which is important for neuronal survival following injury. Thus, the involvement of microglia in both neuroprotection and neurodegeneration cannot be ignored (Heales et al. 2004). We used the sciatic nerve crush (SNC) model to study how modulation of neuroinflammatory changes (microglial activation) may influence the affected neurons and their recovery in young and old rats under the influence of immunosuppressants like FK-506. FK-506 delayed or suppressed expression of MHC-II in microglia not only per cell but also by number of cells expressing them (Saxena et al. 2007). Enhanced, prominent, and chronic microglial activation in the vicinity of both the damaged and normal neurons stimulates neurons to undergo apoptosis (Patro and Patro 2004), and a modulator of neuroinflammation extends increased recovery and survival of neurons (Saxena et al. 2007). This process is impaired in old rats. Injury in old rats has revealed that the surveillance phenotype is different than in the young. In old rats, they have less elaborate and number of processes and reduced motility. Following injury, they present inflammatory response that continues comparatively for a longer duration. The activated microglia in senile tissue could not be influenced by FK-506, and the recovery following SNC was significantly poor (Patro et al. 2008).

6 Priming of Microglia

Microglia live long and are active throughout their life. Aging brings in marked phenotypic and functional changes including cellular dystrophy, impaired phagocytosis, restricted mobility, exaggerated response to inflammatory stimuli (Mosher and Wyss-Coray 2014), and alterations in gene expression (Galatro et al. 2017) in microglia. Such “primed” or “senescent” microglia are considered as causative factors in age-associated cognitive decline and neurodegenerative disorders (Tay

et al. 2017; Angelova and Brown 2019; Niraula et al. 2017; Rawji et al. 2016; Swenson et al. 2019). These cells are exposed to oxidative stress, DNA damage, and age-associated inflammatory insult and finally get primed. Like the macrophages, the microglia, depending on the signals they receive, get converted to M1 (pro-inflammatory) and M2 (phagocytic) states (Ransohoff 2016). However, such distinctive classification of microglia in the CNS is difficult due to the complex environment of the brain. As the terms M1 and M2 were developed through in vitro studies, Ransohoff (2016) has argued that this kind of classification may be dropped with respect to the in vivo condition. Wes et al. (2016) argued that the activation state should be described based on the stimuli that induce the reactive state in the microglia.

Transcriptional and functional changes with age in microglia transform them to a "primed" state. Such cells have elevated levels of pro-inflammatory cytokines like TNFα, IL-1β, and IL-6. With any immune challenge, they become hyperactivated. They also have a reduced phagocytic activity (Mosher and Wyss-Coray 2014). Priming could be classic or alternative. Classic priming is toxic and involves IFN-γ, whereas alternative priming is considered to be neuroprotective and involves IL-4 and IL-13 (Hickman et al. 2013, 2018; Angelova and Brown 2019). During inflammatory response to stimuli, the primed microglia present uncontrolled and heightened response (Perry and Holmes 2014; Cunningham et al. 2005). Primed microglia under normal aging present higher antigen presentation and upregulation of MHC-II, CD86, and CIITA (Godbout et al. 2005; Frank et al. 2006; Sharma et al. 2016).

Priming of microglia was earlier described in many neurodegenerative disorders like multiple sclerosis, Alzheimer's disease, murine ME7 model of the Prion's disease, etc. (Luo et al. 2010). There are clear indications that the primed/sensitized microglia also reside in the aged brain (Godbout and Johnson 2006; Sparkman and Johnson 2008; Bilbo 2010; Streit et al. 2014; Barrientos et al. 2015). Such aging changes in microglia have been suggested to shift their function from physiological to pathological by diminution of their neuroprotective functions, susceptibility to neurotoxicity, and dysregulated responses to signals (Flanary et al. 2007; Streit et al. 2008; Luo et al. 2010; Jurgens and Johnson 2012). Brain aging causes low-level chronic inflammation including long-lasting elevations of pro-inflammatory cytokines in the hippocampus resulting in memory impairments (Barrientos et al. 2009, 2015). Such primed microglia in the aged brain makes them to be over-responsive to small stimuli that are otherwise well controlled in the young brain. When microglia are deregulated, their overactivation could be neurotoxic contributing to neurodegeneration. The destructive roles of activated microglia in the aged neurodegenerative brain possibly result from age-associated microglial senescence with a characteristic morphological feature described as dystrophy (Streit et al. 2004, 2008, 2014).

LPS administration to adult rats results in systemic immune challenge, induced microglial priming following morphological changes like ameboid, and deramified phenotypes. Such a change is also indicative of cognitive and behavioral changes in treated rats (Singh et al. 2017; Sarkar et al. 2019). Similar earlier studies also

reported much elevated and sustained neuroinflammation following LPS injection, chronic illness, and depression (Godbout et al. 2005). Minocycline treatment to LPS-infused rats effectively restricts neuroinflammation (Sharma et al. 2016). However, Sparkman and Johnson (2008) reported low inflammatory response with higher levels of pro-inflammatory cytokine expression in aging brain.

Microglia are long-lived (Weinberg 2008), and hence the aging changes in the physiology of these cells are expected to influence neuronal health. Thus, they are expected to have a major involvement in aging-associated neurodegenerative diseases. Microglial senescence is being examined to be both a cause and effect of the process. Microglia are the major component of the immune system of the brain. With age, the cytokine signature of the microglia changes to a pro-inflammatory state with a decline in their scavenging (phagocytic) properties, thus contributing to the onset and progression of the neurodegenerative diseases (Streit et al. 2004). Such senescent microglia express most pro-inflammatory cytokines, chemokines, ROS, growth factors, proteases, and so on. All these molecules negatively influence the neurons in their vicinity and exaggerate aging changes and neurodegenerative disease (Chinta et al. 2015).

7 Lipofuscin in Aging Microglia

As age advances, the microglia tend to accumulate debris in their soma like the neurons do. Senile microglia tend to be laden with lipofuscin, lipid droplets, phagocytic inclusions including synaptic remnants, and more (Tremblay et al. 2016; Marschallinger et al. 2020). In the cerebellar white matter, microglia have also been identified to accumulate myelin fragments and presented lysosomal overload with age (Safaiyan et al. 2016). This was not consistent in the gray matter. Burns et al. (2020) have reported deposition of proteins involved in autophagy, catabolic process, mitochondrial dysfunction, lysosomal degradation, and others.

Decades back, Hasan et al. (1974) and Patro et al. (1988) conceptualized that microglia play a pivotal role in the removal of lipofuscin from senile neurons. The presence of lipofuscin in microglia was interpreted as a scavenging role rather than considering the possibility that microglia themselves may also be accumulating the pigment. In addition, the accumulation of lipofuscin in nonneuronal cells in the brain continued to be debatable. The presence of microglia in the proximity of lipofuscin laden neurons was considered to be for possible transfer of the pigment by exocytosis from neurons to the microglia (Hasan et al. 1974). However, the presence of the pigment in microglia far away from the neuron may only indicate formation of aging pigment in the microglia themselves more so that the microglia at old age present lower antioxidant defense and this supports the proposition (Vida et al. 2017). Bisht et al. (2016) using flow cytometry identified two different sets of microglia in the aged brain. On one side were low-scattered microglia that had no lipofuscin, while on the other side high-scattered cells with significant deposits of the pigment. The lipofuscin laden microglia had increased ROS and were pro-inflammatory with poor phagocytic activity. These are the dystrophic macrophage phenotype of microglia.

We evaluated lipofuscin accumulation in microglia of senescent rat brain. Microglia in the senescent brain (24–30-month-old rats) contained significantly high amount of lipofuscin, near and far away from the cortex and neurons. Pigment laden microglia were seen at least in three different sites of the brain. Those in proximity of neurons may be considered to help in the removal of the pigment from the neurons. The microglia in the site of lipofuscin scattered in the matrix (possibly leftover debris from the degenerated neurons) performing phagocytosis and the microglia away from the neurons clearly depicting the microglial cells do accumulate the pigment with age (Kushwaha et al. 2018). Our study also established clearly that lipofuscin deposits are not restricted to dystrophic or dysfunctional microglia as they are seen even in intact cells at younger age (18-month-old rats). It is difficult to explain the functional variations in lipofuscin accumulation and the rest of the microglia. It is, however, well established that lipofuscin accumulation beyond a limit influences cellular function irrespective of cell type.

8 ROS and Aging Microglia

Increase in ROS is one of the profound changes in the aging brain. This is also true for most neurodegenerative disorders. Microglia are considered as the main source for oxidative and inflammatory products in the brain. Excessive production of superoxide anions, hydroxyl radicals, and lipid peroxides produced by microglia can be hazardous to the nearby neurons through neurotoxicity or by pro-inflammatory response via protein kinase C (PKC), mitogen activated protein kinase (MAPK), and nuclear factor kappa B (NFkβ) activation (Brown and Griendling 2015). In most age-related neurodegenerative diseases, the presence of activated microglia is believed to produce ROS via NOX (Bordt and Polster 2014). Higher NOX activity has also been correlated with cognitive impairment (Ansari and Scheff 2011). We may thus summarize that increased ROS production in microglia contributes to neurodegenerative disorders and cognitive impairment.

With advancing age or under injury/stressed conditions, NOX and ROS levels are high in the CNS (Zhang et al. 2016). Simultaneously, there is a decline in free radical scavenging mechanism and antioxidant defense in the microglia and the brain in general (Njie et al. 2012; Von Bernhardi et al. 2015). We yet need to understand whether there is higher microglial response to injury at senescence or the aged neurons become more susceptible to ROS leading to neuroinflammation.

9 Aging and Immunoinhibitory Signaling

Microglia are caretakers and guardians of the more sensitive neurons they guard. We understand that while neurons are hypersensitive, microglia react fast to any environmental stress or injury to the neurons. It thus becomes critical to know how the microglia respond as age advances. Microglia in normal conditions are tightly regulated and produce ROS, inflammatory cytokines, and other metabolic

by-products which could be neurotoxic when secreted in higher concentrations. Such regulation is achieved largely by microglial inhibitory receptors that are essential, both, to prevent generation of unwanted inflammation and slow down (even stop) inflammatory response to injury once the neuron or its environment recovers. However, unfortunately, as age advances, the inhibitory receptors that maintain microglial quiescence present a remarkable decline in their ability to do so.

In younger brains, the neurons express and secrete CD200. CD200 in the neurons are stable even on the cell surface. Microglia and macrophages in turn express CD200R1 (the receptor to CD200) that is believed to downregulate their immune response helping in maintaining microglial quiescence. As age advances, the neurons become deficient of CD200 leading to several aging changes in relation to microglia like microglial activation, T cell infiltration, impaired long-term potentiation (LTP), disruption of BBB, and an exaggerated response to any stimuli including injury and disease (Costello et al. 2011; Denieffe et al. 2013; Ritzel et al. 2015, 2016). The decline of CD200 level with aging has also been established (Shrivastava et al. 2012). Such decline in anti-inflammatory responses and related increase in inflammation becomes detrimental to learning and memory (Wang et al. 2011). Regulation of CD200 and CD200R1 expression in neurons and microglia, respectively, in normal aging and AD brain has rendered several positive effects like decreased microglial activation and improved upon age or LPS-associated LTP deficits. Similarly, CD200 infusion improved the pathophysiology induced by Aβ (Cox et al. 2012; Lyons et al. 2012). The chemokine fractalkine CX3CL on neurons and its receptor (CX3CR1) on the microglia behave like CD200 and its receptor. This fractalkine essentially is involved in synaptic function and cognition (Wynne et al. 2010). An age-associated decline in expression of its receptor induces neuroinflammatory changes that impair cognitive function in the aged brain.

10 Phagocytosis in Aging Brain

Microglia in young and aged brain present age-related changes in their function. Phagocytosis is an essential function of microglia in the CNS. In several studies, an age-associated decline in phagocytic activity as well as endocytosis has been reported.

Microglia present an age-associated decline in their phagocytic ability, by almost 50% in the old (Njie et al. 2012) and in AD models of mice (Orre et al. 2014). However, aging does not influence the ability of microglia to uptake bacterial bioparticles (Lynch et al. 2010). Adherence, internalization, digestion, etc. are stages of phagocytosis. It is reported that aging differentially affects each of these steps, dependent on the nature of the substrate. For example, while with aging microglial ability to phagocytose myelin was enhanced, there was a reduction in myelin's susceptibility. This clearly indicates that both extrinsic and intrinsic factors regulate impairment of phagocytosis by microglia (Gitik et al. 2011). Even molecules like CD47 in healthy cells that prevent any phagocytosis could also influence microglia's ability to phagocytose. We have limited idea as to what happens with the expression

of such molecules as age advances. The phagocytic potential of a cell is dependent upon the substrate that initiates microglial activation. One of the examples could be phagocytosis of α-synuclein oligomers that are associated with the TNFα secretion. An age-associated increase in microglial TNFα production is evident. However, our understanding on the role of TNFα overexpression in microglial phagocytosis needs detailed studies (Bliederhaeuser et al. 2016). Overexpression of anti-inflammatory molecules, such as IL-10, is also believed to suppress microglial phagocytosis (Chakrabarty et al. 2015).

11 Microglial Functional Phenotyping with Aging

Over the years, as the brain ages, it is continuously exposed to several internal and external factors that stimulate the immune system of the CNS. The factors could be continued or repeated exposure to infections like a virus, presence of antigenic modified endogenous proteins, cellular debris, and others. The low level and controlled presence of inflammation with aging to the senile state is described as "inflammaging" (Franceschi et al. 2017; Brawek et al. 2021). In our studies with early life exposure to protein malnutrition, viral or bacterial exposures of rat pups initiated microglial activation to states of various grades of neuroinflammation. Such inflammatory state remains prevalent even at advanced age of these rats. This also leads to elevated presence of ROS and associated damage (Sinha et al. 2019, 2020).

Age-related changes are evident in microglial function in 9–12-month-old mice, i.e., young adults, where microglia presented insignificant changes like an increase in soma volume and retraction of processes but had a significantly low mobility suggesting impaired surveillance function (Hefendehl et al. 2014; Bayliak et al. (2021). At about 12 months of age, considered to be the middle age, the brain presents high oxidative stress and aging-related changes. The "dark" microglial cells described earlier appear by 9 months of age in mice (Bisht et al. 2016). We have much to learn on these dark microglia. "Dark" microglia have been seen in the chronically stressed, diseased, or aging brain in most of the brain regions (Bisht et al. 2016). They are also abundantly present in the developing brain (Stratoulias et al. 2019) extensively participating in the process of synaptogenesis (St-Pierre et al. 2020) in both rodents and human (Calì et al. 2019; Uranova et al. 2018).

The microglia in healthy young and middle-aged animals presented similar levels of expression as far as pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) are concerned. However, upon any insult or challenge, the microglia in middle-aged animals express high levels of pro-inflammatory cytokines (Nikodemova et al. 2016). This has been related to the differential expression of P2 receptors with age and gender, e.g., P2X1 and P2X3 receptors are expressed more as age advanced (Crain et al. 2009). When the animals were housed in enriched environment as they reached middle age, the microglia in their brains were found normal with less incidence of deramification of process, downregulated immune expression, and impaired behavioral outcome (McMurphy et al. 2018). It is thus expected that proper intervention at middle age is expected to control microglial overreactivity, delay

onset of age-related changes, and downregulate age-related hyperactivity of immune regulatory system.

Chronic low-grade inflammatory state is a hallmark of aged brain with hyper expression of pro-inflammatory cytokines, MHC-II, and complement receptors. This is additionally associated with a downregulation of anti-inflammatory genes (Cribbs et al. 2012). The aged brains have two different sets of microglia, the aged and chronically activated microglia. The latter present extensive NF-kβ signaling (Holtman et al. 2015). Several studies have indicated that aged microglia are more prone to inflammatory changes and are primed cells. Aging-induced increased expression of pro-inflammatory cytokines in the brain is being related to age-associated decline in memory and cognition (Youm et al. 2013). Deczkowska et al. (2018) described a set of microglia called disease-associated microglia (DAM) with downregulation of a set of genes (*P2ry12/P2ry13*, *Cx3cr1*, and *Tmem119*) responsible for homeostatic function of microglia. Such cells present a heightened lysosomal activity, phagocytic functions, and several factors involved with onset of AD. The number of such microglia increases with age. DAM cells are believed to accumulate and then remove the damaged content of the aged brain. Brains of mouse models of AD, amyotrophic lateral sclerosis, and tauopathy all have DAM microglia. Similarly, AD brain tissues also have DAM cells seen in close approximation to plaques. The aging brain accumulates such cells to the tune of 3% of all microglial cells in senile mice (Keren-Shaul et al. 2017).

Krasemann et al. (2017) described yet another type of microglia in neurodegenerative disease brain and called them "microglial neurodegenerative phenotype" (MGnD). Like DAM, the MGnD show suppressed homeostatic genes (*P2ry12*, *Tmem119*, *Olfm13*, *Cst1r*, *Rhob*, *Cx3cr1*, *Tgfb1*, *Maf2a*, *Mafb*, *Sall1*) and upregulated inflammatory molecules (Apoe, Spp1, Itgax, Ax1, csf1, etc.). MGnD are defensive cells and respond to neuronal injury. The molecular status of these microglia responds well to any neuronal damage via phagocytosis, chemotaxis, cytokine release, etc.; DAM and MGnD differ at transcription level (Marschallinger et al. 2020).

About half of the microglia in the senile brain accumulate phospho- and glycolipids. Similarly, microglia in aging and senile human and rat brain accumulate lipofuscin granules (discussed vide supra). These lipid laden microglia in aging and senile brain have been named as "lipid droplet-accumulating microglia" (LDAM) and present age-related functional decline. Higher extracellular lipid content, inflammation and associated hike in ROS, intracellular metabolic changes, inflammation, and stress all contribute to the formation of LDAM cells (Rambold et al. 2015; Hu et al. 2017). LDAM microglia have been reported to have higher oxidative damage, express excessive cytokines like IL-6 and CCL-3, and have low efficacy phagocytosis (Marschallinger et al. 2020). These microglia are dysfunctional microglia and constitute larger part of aged microglia and play a role in increased ROS generation. It has been proposed that age-associated challenges initially cause elevated ROS that initiate lipid droplet formation but in turn such lipid droplets in autocatalytic manner themselves induce ROS generation and disturb the intracellular ROS load (Marschallinger et al. 2020).

Upregulation of CD22 in aging microglia impairs phagocytosis. Blocking of CD22 promotes debris clearance in the aging brain and subsequently downregulates inflammatory factors and improves cognitive ability in aged mice (Pluvinage et al. 2019). The process of phagocytosis directly impairs cognitive functions in the aging brain. Therapeutic intervention on cognitive dysfunction with age can be addressed through CD22 blockers.

Microglial population is believed to remain almost unchanged during aging process (Askew et al. 2017). In the human and rat brain, microglial heterogeneity in terms of both morphological and functional phenotypes is prominent (Streit et al. 2020). Microglia in the senescent brain are mostly dystrophic, presenting deramified, gnarled, ameboid, and fragmented or degenerating states (Streit et al. 2004). The human senescent brain has always been described to present distinctly higher population of such dystrophic cells. This could be explained based on the length of the life span, chronic exposure to environmental hazards and mental states of non-symptomatic neuropathological effects (Streit et al. 2014, 2020). Prominence of phagocytosis, surveillance, ramification, and process motility at younger life gets transformed to microglia with larger soma size, lipofuscin accumulation, decline in surveillance, deramification, and dystrophy as age advances to senescence. Microglia in the senescent brain could both be dystrophic, characterized by their impaired surveillance, disturbed process mobility, and a reduction in phagocytosis, and reactive presenting exaggerate microglial response to injury or even minor tissue damage.

12 Counteracting Aging of Microglia

The nicotinic acetylcholine receptor of α7 subtype (α7nAChRs) abundantly expressed in the brain plays a major role in responding to pro-inflammatory stimuli (Moretti et al. 2014). α7nAChRs are found in all major cell types, such as neurons, microglia, and astrocytes (Moretti et al. 2014). Upon activation, α7nAChRs attenuate production of inflammatory cytokines like IL-1β, IL-6, or TNFα (Zhang et al. 2017; DeJonge and Ulloa 2007; Cortes et al. 2017). A decrease in level of α7nAChRs due to neuroinflammation in the aging brain contributes to the process of accumulation of Aβ1-42.

A pro-inflammatory state in brain aging is believed to promote stroke (Lee et al. 2014). Similarly, inflammation may contribute to persistent neuropathologic pain in older people (Pickering et al. 2016). Neuroinflammation is one of the strong factors in manifestation of age-related neurodegenerative disorders like AD and PD (Hickman et al. 2018). Several studies have established that activation of α7nAChRs exerts beneficial effects against stroke (Guan et al. 2015), neuropathic pain (Ji et al. 2019), and attenuation of AD- and PD-associated brain dysfunction (Lykhmus et al. 2015) by suppressing neuroinflammation. Therapeutic activation of α7nAChRs has thus been recorded to cure neurodegenerative disorders mediated by neuroinflammation (Foucault-Fruchard and Antier 2017).

13 Microglia Repopulation in the Aging Brain

We have now understood that reactive microglia induce tissue damage and potentially contribute to the onset of neurodegenerative diseases. Normalizing or selectively depleting activated microglia with immunotherapy may help (Elmore et al. 2018; Han et al. 2018). Specifically targeting activated microglia has been achieved by pharmacological inhibition or genetic targeting. Several modes to achieve this have been reviewed in detail by Han et al. (2018).

Elimination of microglia in AD brain resulted in a reduction in neuron loss, improved memory, and prevention of disease progression (Asai et al. 2015; Spangenberg et al. 2016). When microglia were depleted pharmacologically in early time point and for a longer duration in mouse models of AD, even plaque deposition and amyloid formation could be avoided (Sosna et al. 2018). Similar results have also been reported like slowing disease progression and increased life expectancy in ALS (Martínez-Muriana et al. 2016), neuropathic pain via reduction of inflammatory cytokines (Lee et al. 2018), and others. It is advocated that depletion be tried only after disease onset (Yang et al. 2018).

Microglial elimination and repopulation are believed to be useful only when altered microglial phenotypes are more prevalent impinging brain function, e.g., during aging. Dystrophic or "primed" microglia in the aging brain have impaired phagocytosis and reduced motility and respond intensely to inflammatory stimuli (Mosher and Wyss-Coray 2014). Renewal of such microglia with pharmacological interventions, e.g., withdrawal of CSFIR inhibition, can stimulate microglial repopulation by replacement with new microglia in 14–21 days (Elmore et al. 2018). Such microglial repopulation helps to improve cognition in aged mice. Aged mice in their study presented improved spatial performance in MWM. The process of repopulation helped to attain microglial cell densities and morphology similar to young adult mice.

With experimental microglial activation (Patro and Patro 2004; Sharma et al. 2016) in the aged brain during chronically activated microglia (Barrientos et al. 2015), neurogenesis declines. Neurogenesis is elevated following microglial elimination in the aged brain but could be restored to the control level after repopulation indicating that microglia dynamically regulate neurogenesis. Aged mice also exhibit notable impairments in LTP which was completely restored to young control level following microglial repopulation, indicating the efficacy of microglial replacement therapy (Elmore et al. 2018).

It is now believed that microglial replacement therapy, i.e., elimination of old microglia and repopulating the brain with new fully differentiated microglia, could be one of the effective interventions in several neurological disorders. However, more detailed studies with animal models on the timing, duration, and frequency of such elimination and repopulation would further rationalize the procedure.

14 Modeling Aging Microglia

We now have increasing evidence in support of involvement of microglia in most neurodegenerative diseases. Neurodegenerative diseases are largely associated with aging. Thus, study of aging in microglia and role of microglia in the aging brain both have gained importance in neuroscience. This aspect has been discussed in detail. Obviously, the aging animals, like rat and mouse, are being used for such studies on aging and senescence in microglia. This is time-consuming, expensive, and practically very difficult if not impossible. Undoubtedly, studying aged microglia in senescent brains is of importance as mimicking the microenvironment of the aging brain in vitro may not be as accurate as it is in vivo. After all, time can't be replaced by anything else. On the other hand, isolating microglia in their healthy state or maintaining them to have a culture of aging or aged microglia is also very difficult. Age of the animal reflects the aging changes in microglia. There are several factors that stimulate biologists to look for experimental models. The major requirements for this have been the ethical issues and cost of maintenance of animals in a well-controlled environment, for the entire life span of the species, in captivity, is also both expensive and impractical. This has encouraged development of several models that replicate the aging microglia to a great extent. The various models and their properties have been listed in Table 20.1. These models have been reviewed by Greenwood and Brown (2021), Angelova and Brown (2019), and Xu et al. (2021).

15 Conclusion

It is clear that microglia with age get converted to divergent phenotypes with diverse functional responsibility. The aged/senescent microglia can safely be considered as a prime causative component of most of the neurodegenerative disorders. Experimental manipulation of such microglia has helped in reviving the cognitive ability of experimental animals at old age. It is also clear that depletion, rejuvenation, and replacement of the reactive, dystrophic, and senescent microglia all have positively influenced age-related cognitive decline. However, the diversity of microglia in the senile brain prevents us from considering to find out suitable marker(s) for senescence (Böttcher et al. 2019). Microglial activated and age-associated phenotypes have also been found responsible for the neuropathology we find with neurodegenerative disorders (Keren-Shaul et al. 2017).

Identification of suitable markers for senescent microglia is very important and will provide opportunities to develop models of microglia suitable to be designated of being responsible for aging changes in the brain per se (Béraud et al. 2013; Angelova and Brown 2019).

The important aspect of this research is that the senescent microglia can be targeted, eliminated, and repopulated with new and surveillant microglia. This and other therapeutic means to combat the negative impact of dystrophic microglia have tremendous translational value in view of the increased human life expectancy and high prevalence of neurodegenerative disorders.

Table 20.1 Modeling aging microglia

	Model	Reference	Effect	Microglia status
1.	Transgenic mice $Ercc1^{\Delta/-}$	Raj et al. (2014)	Accelerated aging through DNA repair deficiency	Age-related changes in microglia
2.	Transgenic mouse $mTerc^{-/-}$	Raj et al. (2015)	Telomere shortening	Microglia exhibit physiological state of senescence and priming
3.	Microglia isolated from neonatal mice and then maintained in culture	Caldeira et al. (2014)	Reduced phagocytic ability, mobility, autophagy; changes in microRNAs and SA-β-galactose activity	Resemble aged microglia but very short-lived (± 16 days)
4.	Treatment of primary microglia culture with dexamethasone	Park et al. (2019)	Increased SA-β-galactosidase activity; upregulation of tumor suppressor genes; dysfunction of phagocytosis; limitations are increased autophagy, low expression of inflammatory markers, and low cytokine release	Simulates chronic stress that microglia experience over time and aging; senescent microglia
5.	Overloading microglia with iron in culture (primary mouse microglia; human and mouse microglia cell lines)	Angelova and Brown (2018a, b, 2019)	Secretory profiles like SASP; increased release of ROS and cytokines (IL-1β, TNF-α, and interferon-γ); decreased rate of proliferation; altered expression of SIRT-1 and Kv.1.3 proteins; change in glutamate release; decreased autophagy; able to induce changes in neuronal cell lines similar to neurodegenerative diseases	Aging microglia with senescence and dystrophic changes
6.	Repeated injections of phorbol myristate into the substantia nigra of rats	Liu et al. (2015)	Increased activity of β-galactosidase and p21 induction	Induce senescent phenotype of microglia
7.	Intracranial injection of LPS in adult rats; perinatal systemic LPS injection (i.p.)	Sharma et al. (2016)	Overexpression of the cascade of inflammatory cytokines	Persistent presence of activated and primed microglia in rat brain

(continued)

Table 20.1 (continued)

	Model	Reference	Effect	Microglia status
8.	Intracranial injection of poly I:C in adult rats; perinatal systemic poly I:C injection (i.p.)	Patro and Patro (2004)	Overexpression of the cascade of inflammatory cytokines	Persistent presence of activated and primed microglia in rat brain

Acknowledgments Department of Biotechnology's support through the DBT National Initiative on Glial Cell Research in Health and Disease is gratefully acknowledged. We are thankful to the Department of Biotechnology, Department of Science and Technology, and the Indian Council of Medical Research, Govt. of India, for their financial support through various grants.

References

Angelova DM, Brown DR (2018a) Altered processing of beta- amyloid in SH-SY5Y cells induced by model senescent microglia. ACS Chem Neurosci 9:3137–3152

Angelova DM, Brown DR (2018b) Model senescent microglia induce disease related changes in alpha-synuclein expression and activity. Biomol Ther 8:67

Angelova DM, Brown DR (2019) Microglia and the aging brain: are senescent microglia the key to neurodegeneration. J Neurochem 151(6):676–688

Ansari MA, Scheff SW (2011) NADPH-oxidase activation and cognition in Alzheimer disease progression. Free Radic Biol Med 51(1):171–178

Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, Wolozin B, Butowsky O, Ikezu T (2015) Depletion of microglia and inhibition of exosome synthesis halt tau propagation. Nat Neurosci 18(11):1584–1593

Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P et al (2017) Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. Cell Rep 18(2):391–405

Bachstetter AD, Ighodaro ET, Hassoun Y, Aldeiri D, Neltner JH, Patel E et al (2017) Rod-shaped microglia morphology is associated with aging in 2 human autopsy series. Neurobiol Aging 100(52):98–105

Barrientos RM, Frank MG, Hein AM, Higgins EA, Watkins LR, Rudy JW et al (2009) Time course of hippocampal IL-1 beta and memory consolidation impairments in aging rats following peripheral infection. Brain Behav Immun 23(1):46–54

Barrientos RM, Kitt MM, Watkins LR, Maier SF (2015) Neuroinflammation in the normal aging hippocampus. Neuroscience 309:84–99

Bayliak MM, Sorochynska OM, Kuzniak OV, Gospodaryov DV, Demianchuk I, Vasylyk YV (2021) Middle age as a turning point in mouse cerebral cortex energy and redox metabolism: modulation by every-other-day fasting. Exp Gerontol 145:111182

Béraud D, Hathaway HA, Trecki J, Chasovskikh S, Johnson DA, Johnson JA et al (2013) Microglial activation and antioxidant responses induced by the Parkinson's disease protein α-synuclein. J Neuroimmune Pharmacol 8(1):94–117

Bilbo SD (2010) Early life infection is a vulnerability factor for aging-related glial alterations and cognitive decline. Neurobiol Learn Mem 94(1):57–64

Bisht K, Sharma KP, Lecours C, Gabriela Sánchez M, El Hajj H, Milior G et al (2016) Dark microglia: a new phenotype predominantly associated with pathological states. Glia 64(5): 826–839

Bliederhaeuser C, Grozdanov V, Speidel A, Zondler L, Ruf WP, Bayer H (2016) Age-dependent defects of alpha-synuclein oligomer uptake in microglia and monocytes. Acta Neuropathol 131(3):379–391

Bordt EA, Polster BM (2014) NADPH oxidase-and mitochondria-derived reactive oxygen species in proinflammatory microglial activation: a bipartisan affair? Free Radic Biol Med 100(76): 34–46

Böttcher C, Schlickeiser S, Sneeboer MA, Kunkel D, Knop A, Paza E et al (2019) Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. Nat Neurosci 22(1):78–90

Brawek B, Skok M, Garaschuk O (2021) Changing functional signatures of microglia along the axis of brain aging. Int J Mol Sci 22(3):1091

Brown DI, Griendling KK (2015) Regulation of signal transduction by reactive oxygen species in the cardiovascular system. Circ Res 116(3):531–549

Burns JC, Cotleur B, Walther DM, Bajrami B, Rubino SJ, Wei R et al (2020) Differential accumulation of storage bodies with aging defines discrete subsets of microglia in the healthy brain. elife 9:57495

Bussian TJ, Aziz A, Meyer CF, Swenson BL, van Deursen JM, Baker DJ (2018) Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. Nature 562(7728): 578–582

Caldeira C, Oliveira AF, Cunha C, Vaz AR, Falcao AS, Fernandes A, Brites D (2014) Microglia change from a reactive to an age-like phenotype with the time in culture. Front Cell Neurosci 8: 152

Calì C, Agus M, Kare K, Boges DJ, Lehväslaiho H, Hadwiger M, Magistretti PJ (2019) 3D cellular reconstruction of cortical glia and parenchymal morphometric analysis from serial block-face electron microscopy of juvenile rat. Prog Neurobiol 183:101696

Chakrabarty P, Li A, Ceballos-Diaz C, Eddy JA, Funk CC, Moore B et al (2015) IL-10 alters immunoproteostasis in APP mice, increasing plaque burden and worsening cognitive behavior. Neuron 85(3):519–533

Chinta SJ, Woods G, Rane A, Demaria M, Campisi J, Andersen JK (2015) Cellular senescence and the aging brain. Exp Gerontol 68:3–7

Conde JR, Streit WJ (2006) Microglia in the aging brain. J Neuropathol Exp Neurol 65(3):199–203

Conrad AT, Dittel BN (2011) Taming of macrophage and microglial cell activation by microRNA-124. Cell Res 21(2):213–216

Coppé JP, Desprez PY, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Patho 5:99–118

Cortes M, Cao M, Liu HL, Moore CS, Durosier LD, Burns P et al (2017) α7 nicotinic acetylcholine receptor signaling modulates the inflammatory phenotype of fetal brain microglia: first evidence of interference by iron homeostasis. Sci Rep 7(1):1–5

Costello DA, Lyons A, Denieffe S, Browne TC, Cox FF, Lynch MA (2011) Long term potentiation is impaired in membrane glycoprotein CD200-deficient mice: a role for toll-like receptor activation. J Biol Chem 286(40):34722–34732

Cox FF, Carney D, Miller AM, Lynch MA (2012) CD200 fusion protein decreases microglial activation in the hippocampus of aged rats. Brain Behav Immun 26(5):789–796

Crain JM, Nikodemova M, Watters JJ (2009) Expression of P2 nucleotide receptors varies with age and sex in murine brain microglia. J Neuroinflamm 6(1):1–6

Cribbs DH, Berchtold NC, Perreau V, Coleman PD, Rogers J, Tenner AJ et al (2012) Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study. J Neuroinflamm 9(1):1–8

Cunningham C, Wilcockson DC, Campion S, Lunnon K, Perry VH (2005) Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. J Neurosci 25(40):9275–9284

Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I (2018) Disease-associated microglia: a universal immune sensor of neurodegeneration. Cell 173(5):1073–1081

DeJonge WJ, Ulloa L (2007) The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. Br J Pharmacol 151(7):915–929
Delage CI, Šimončičová E, Tremblay MÈ (2021) Microglial heterogeneity in aging and Alzheimer's disease: is sex relevant? J Pharmacol Sci 246(3):169–181
Denieffe S, Kelly RJ, McDonald C, Lyons A, Lynch MA (2013) Classical activation of microglia in CD200-deficient mice is a consequence of blood brain barrier permeability and infiltration of peripheral cells. Brain Behav Immun 34:86–97
Elmore MR, Hohsfield LA, Kramár EA, Soreq L, Lee RJ, Pham ST et al (2018) Replacement of microglia in the aged brain reverses cognitive, synaptic, and neuronal deficits in mice. Aging Cell 17(6):12832
Esiri MM (2007) The interplay between inflammation and neurodegeneration in CNS disease. J Neuroimmunol 184(1–2):4–16
Fendrick SE, Xue QS, Streit WJ (2007) Formation of multinucleated giant cells and microglial degeneration in rats expressing a mutant cu/Zn superoxide dismutase gene. J Neuroinflamm 4(1):1–12
Flanary BE, Streit WJ (2004) Progressive telomere shortening occurs in cultured rat microglia, but not astrocytes. Glia 45(1):75–88
Flanary BE, Sammons NW, Nguyen C, Walker D, Streit WJ (2007) Evidence that aging and amyloid promote microglial cell senescence. Rejuvenation Res 10(1):61–74
Foucault-Fruchard L, Antier D (2017) Therapeutic potential of alpha 7 nicotinic receptor agonists to regulate neuroinflammation in neurodegenerative diseases. Neural Reg Res 12(9):1418–1421
Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S (2017) Inflammaging and Garb-aging. Trends Endocrinol Metab 28(3):199–212
Frank MG, Barrientos RM, Biedenkapp JC, Rudy JW, Watkins LR, Maier SF (2006) mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. Neurobiol Aging 27(5):717–722
Galatro TF, Holtman IR, Lerario AM, Vainchtein ID, Brouwer N, Sola PR et al (2017) Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. Nat Neurosci 20(8):1162–1171
Gitik M, Liraz-Zaltsman S, Oldenborg PA, Reichert F, Rotshenker S (2011) Myelin down-regulates myelin phagocytosis by microglia and macrophages through interactions between CD47 on myelin and SIRPα (signal regulatory protein-α) on phagocytes. J Neuroinflamm 8(1):1–11
Godbout JP, Johnson RW (2006) Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. Neuro Clin 24(3):521–538
Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW (2005) Exaggerated neuroinflammation and sickness behavior in aged mice after activation of the peripheral innate immune system. FASEB J 19(10):1329–1331
Graeber MB, Tetzlaff W, Streit WJ, Kreutzberg GW (1988) Microglial cells but not astrocytes undergo mitosis following rat facial nerve axotomy. Neurosci Lett 85(3):317–321
Greenwood EK, Brown DR (2021) Senescent microglia: the key to the aging brain. Int J Mole Sci 22(9):4402
Guan YZ, Jin XD, Guan LX, Yan HC, Wang P, Gong Z et al (2015) Nicotine inhibits microglial proliferation and is neuroprotective in global ischemia rats. Mol Neurobiol 51(3):1480–1488
Han J, Zhu K, Zhang XM, Harris RA (2018) Enforced microglial depletion and repopulation as a promising strategy for the treatment of neurological disorders. Glia 67(2):217–231
Hasan M, Glees P, Spoerri PE (1974) Dissolution and removal of neuronal lipofuscin following dimethylaminoethyl p-chlorophenoxyacetate administration to Guinea pigs. Cell Tissue Res 150(3):369–375
Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25(3):585–621
Heales SJ, Lam AA, Duncan AJ, Land JM (2004) Neurodegeneration or neuroprotection: the pivotal role of astrocytes. Neurochem Res 29(3):513–519

Hefendehl JK, Neher JJ, Sühs RB, Kohsaka S, Skodras A, Jucker M (2014) Homeostatic and injury-induced microglia behavior in the aging brain. Aging Cell 13(1):60–69
Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang LC, Means TK et al (2013) The microglial sensome revealed by direct RNA sequencing. Nat Neurosci 16(12):1896–1905
Hickman S, Izzy S, Sen P, Morsett L, El Khoury J (2018) Microglia in neurodegeneration. Nat Neurosci 21(10):1359–1369
Holtman IR, Raj DD, Miller JA, Schaafsma W, Yin Z, Brouwer N et al (2015) Induction of a common microglia gene expression signature by aging and neurodegenerative conditions: a co-expression meta-analysis. Acta Neuropathol Commun 3(1):1–8
Hristovska I, Pascual O (2015) Deciphering resting microglial morphology and process motility from a synaptic prospect. Front Integ Neurosci 9:73
Hu X, Xu B, Ge W (2017) The role of lipid bodies in the microglial aging process and related diseases. Neurochem Res 42(11):3140–3148
Hukkelhoven CW, Steyerberg EW, Rampen AJ, Farace E, Habbema JD, Marshall LF, Murray GD, Mass IR (2003) Patient age and outcome following severe traumatic brain injury: an analysis of 5600 patients. J Neurosurg 99(4):666–673
James TJ, Sharma SP, Gupta SK, Patro IK (1992) 'Dark' cell formation under protein malnutrition: process of conversion and concept of' semi-dark' type Purkinje cells. Indian J Exp Biol 30(6): 470–473
Ji L, Chen Y, Wei H, Feng H, Chang R, Yu D (2019) Activation of alpha7 acetylcholine receptors reduces neuropathic pain by decreasing dynorphin A release from microglia. Brain Res 1715: 57–65
Jurgens HA, Johnson RW (2012) Dysregulated neuronal-microglial cross-talk during aging, stress and inflammation. Exp Neurol 233(1):40–48
Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK et al (2017) A unique microglia type associated with restricting development of Alzheimer's disease. Cell 169(7):1276–1290
Klegeris A, Choi HB, McLarnon JG, McGeer PL (2007) Functional ryanodine receptors are expressed by human microglia and THP-1 cells: their possible involvement in modulation of neurotoxicity. J Neurosci Res 85(10):2207–2215
Korotzer AR, Pike CJ, Cotman CW (1993) β-Amyloid peptides induce degeneration of cultured rat microglia. Brain Res 624(1–2):121–125
Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R (2017) The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. Immunity 47(3):566–581
Kullberg S, Aldskogius H, Ulfhake B (2001) Microglial activation, emergence of ED1-expressing cells and clusterin upregulation in the aging rat CNS, with special reference to the spinal cord. Brain Res 899(1–2):169–186
Kushwaha SS, Patro N, Patro IK (2018) A sequential study of age-related lipofuscin accumulation in hippocampus and striate cortex of rats. Ann Neurosci 25(4):223–233
Lee Y, Lee SR, Choi SS, Yeo HG, Chang KT, Lee HJ (2014) Therapeutically targeting neuroinflammation and microglia after acute ischemic stroke. Biomed Res Int 2014:297241
Lee S, Shi XQ, Fan A, West B, Zhang J (2018) Targeting macrophage and microglia activation with colony stimulating factor 1 receptor inhibitor is an effective strategy to treat injury-triggered neuropathic pain. Mol Pain 14:1744806918764979
Liu L, Luo XG, Yu HM, Feng Y, Ren Y, Yin YF, Shang H, He ZY (2015) Repeated intra-nigrostriatal injection of phorbol myristate acetate induces microglial senescence in adult rats. Mol Med Rep 12:7271–7278
Luo XG, Ding JQ, Chen SD (2010) Microglia in the aging brain: relevance to neurodegeneration. Mol Neurodegener 5(1):1–9
Lykhmus O, Voytenko L, Koval L, Mykhalskiy S, Kholin V, Peschana K et al (2015) α7 nicotinic acetylcholine receptor-specific antibody induces inflammation and amyloid β42 accumulation in the mouse brain to impair memory. PLoS One 10(3):0122706

Lynch AM, Murphy KJ, Deighan BF, O'Reilly JA, Gun'ko YK, Cowley TR et al (2010) The impact of glial activation in the aging brain. Aging Dis 1(3):262–278
Lyons A, Downer EJ, Costello DA, Murphy N, Lynch MA (2012) Dok2 mediates the CD200Fc attenuation of Aβ-induced changes in glia. J Neuroinflamm 9(1):107
Marschallinger J, Iram T, Zardeneta M, Lee SE, Lehallier B, Haney MS et al (2020) Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. Nat Neurosci 23(2):194–208
Martínez-Muriana A, Mancuso R, Francos-Quijorna I, Olmos-Alonso A, Osta R, Perry VH et al (2016) CSF1R blockade slows the progression of amyotrophic lateral sclerosis by reducing microgliosis and invasion of macrophages into peripheral nerves. Sci Rep 6(1):1–3
Masuda T, Sankowski R, Staszewski O, Prinz M (2020) Microglia heterogeneity in the single-cell era. Cell Rep 30(5):1271–1281
McMurphy T, Huang W, Queen NJ, Ali S, Widstrom KJ, Liu X et al (2018) Implementation of environmental enrichment after middle age promotes healthy aging. Aging (Albany NY) 10(7): 1698–1721
Miller KR, Streit WJ (2007) The effects of aging, injury and disease on microglial function: a case for cellular senescence. Neuron Glia Biol 3(3):245–253
Moraga A, Pradillo JM, García-Culebras A, Palma-Tortosa S, Ballesteros I, Hernández-Jiménez M (2015) Aging increases microglial proliferation, delays cell migration, and decreases cortical neurogenesis after focal cerebral ischemia. J Neuroinflamm 12(1):1–2
Moretti M, Zoli M, George AA, Lukas RJ, Pistillo F, Maskos U (2014) The novel α7β2-nicotinic acetylcholine receptor subtype is expressed in mouse and human basal forebrain: biochemical and pharmacological characterization. Mol Pharmacol 86(3):306–317
Morris GP, Clark IA, Zinn R, Vissel B (2013) Microglia: a new frontier for synaptic plasticity, learning and memory, and neurodegenerative disease research. Neurobiol Learn Mem 105:40–53
Mosher KI, Wyss-Coray T (2014) Microglial dysfunction in brain aging and Alzheimer's disease. Biochem Pharmacol 88(4):594–604
Nakanishi H, Wu Z (2009) Microglia-aging: roles of microglial lysosome-and mitochondria-derived reactive oxygen species in brain aging. Behav Brain Res 201(1):1–7
Nikodemova M, Small AL, Kimyon RS, Watters JJ (2016) Age-dependent differences in microglial responses to systemic inflammation are evident as early as middle age. Physiol Genomics 48(5): 336–344
Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318
Niraula A, Sheridan JF, Godbout JP (2017) Microglia priming with aging and stress. Neuropsychopharmacol Rev 42(1):318–333
Njie EG, Boelen E, Stassen FR, Steinbusch HW, Borchelt DR, Streit WJ (2012) Ex vivo cultures of microglia from young and aged rodent brain reveal age-related changes in microglial function. Neurobiol Aging 33(1):191–195
Ogura KI, Ogawa M, Yoshida M (1994) Effects of ageing on microglia in the normal rat brain: immunohistochemical observations. Neuroreport 5(10):1224–1226
Orre M, Kamphuis W, Osborn LM, Melief J, Kooijman L, Huitinga I et al (2014) Acute isolation and transcriptome characterization of cortical astrocytes and microglia from young and aged mice. Neurobiol Aging 35(1):1–14
Palace J (2007) Inflammation versus neurodegeneration: consequences for treatment. J Neurol Sci 259(1–2):46–49
Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P et al (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333(6048):1456–1458
Park MJ, Park HS, You MJ, Yoo J, Kim SH Kwon MS (2019) Dexamethasone induces a specific form of ramified dysfunctional microglia. Mol Neurobiol 56:1421–1436
Patro N, Patro IK (2004) Effect of immuneactivator (poly I:C) on the rat cerebral cortex. J Tissue Res 4:71–74

Patro IK, Sharma SP, Patro N (1988) Neuronal lipofuscin: its formation and reversibility. Rev Life Sci 8:95–120

Patro IK, Pathak S, Patro N (2005) Central response to peripheral nerve injury: role of non-neuronal cells. In: Thakur MK, Prasad S (eds) Molecular and cellular neurobiology. Narosa, Delhi, pp 217–233

Patro I, Saxena K, Tiwari S, Patro N (2008) FK506 helps motor coordination recovery following sciatic nerve transection in the young but not in the senile rats. Int J Neuroprotect Neuroregen 4: 145–150

Patro N, Kushwaha SS, Patro IK (2010) Ageing of microglia: does it influence neuroprotection? Proc Nat Acad Sci 80:14–24

Patro N, Singh K, Patro IK (2013) Differential microglial and astrocytic response to bacterial and viral infection in the developing hippocampus of neonatal rats. Ind J Exp Biol 51(8):606–614

Patro IK, Nagayach A, Sinha S, Patro N (2016) General physiology and pathophysiology of microglia during neuroinflammation. In: Jana N, Basu A, Tandon PN (eds) Inflammation: the common link in brain pathologies, 1st edn. Springer, pp 17–42

Perry VH, Holmes C (2014) Microglial priming in neurodegenerative disease. Nat Rev Neurol 10(4):217–224

Perry VR, Matyszak MK, Feam S (1993) Altered antigen expression of microglia in the aged rodent brain. Glia 7(1):60–67

Perry VH, Nicoll JA, Holmes C (2010) Microglia in neurodegenerative disease. Nat Rev Neurol 6(4):193–201

Pickering G, Marcoux M, Chapiro S, David L, Rat P, Michel M et al (2016) An algorithm for neuropathic pain management in older people. Drugs Aging 33(8):575–583

Pluvinage JV, Haney MS, Smith BA, Sun J, Iram T, Bonanno L (2019) CD22 blockade restores homeostatic microglial phagocytosis in ageing brains. Nature 568(7751):187–192

Prinz M, Priller J (2014) Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. Nat Rev Neurosci 15(5):300–312

Raivich G, Jones LL, Werner A, Blüthmann H, Doetschmann T, Kreutzberg GW (1999) Molecular signals for glial activation: pro-and anti-inflammatory cytokines in the injured brain. Acta Neurochir Suppl 73:21–30

Raj DDA, Jaarsma D, Holtman IR, Olah M, Ferreira FM, Schaafsma W, Brouwer N et al (2014) Priming of microglia in a DNA-repair deficient model of accelerated aging. Neurobiol Aging 35: 2147–2160

Raj DDA, Moser J, van der Pol SMA, van Os RP, Holtman IR, Brouwer N et al (2015) Enhanced microglial pro-inflammatory response to lipopolysaccharide correlates with brain infiltration and blood-brain barrier dysregulation in a mouse model of telomere shortening. Aging Cell 14: 1003–1013

Rambold AS, Cohen S, Lippincott-Schwartz J (2015) Fatty acid trafficking in starved cells: regulation by lipid droplet lipolysis, autophagy, and mitochondrial fusion dynamics. Dev Cell 32(6):678–692

Ransohoff RM (2016) A polarizing question: do M1 and M2 microglia exist? Nat Neurosci 19(8): 987–991

Rawji KS, Mishra MK, Michaels NJ, Rivest S, Stys PK, Yong VW (2016) Immunosenescence of microglia and macrophages: impact on the ageing central nervous system. Brain 139(3): 653–661

Ritzel RM, Patel AR, Pan S, Crapser J, Hammond M, Jellison E et al (2015) Age-and location-related changes in microglial function. Neurobiol Aging 36(6):2153–2163

Ritzel RM, Crapser J, Patel AR, Verma R, Grenier JM, Chauhan A et al (2016) Age-associated resident memory CD8 T cells in the central nervous system are primed to potentiate inflammation after ischemic brain injury. J Immunol 196(8):3318–3330

Rogers JT, Morganti JM, Bachstetter AD, Hudson CE, Peters MM, Grimmig BA et al (2011) CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J Neurosci 31(45):16241–16250

Safaiyan S, Kannaiyan N, Snaidero N, Brioschi S, Biber K, Yona S et al (2016) Age-related myelin degradation burdens the clearance function of microglia during aging. Nat Neurosci 19(8): 995–998

Santambrogio L, Belyanskaya SL, Fischer FR, Cipriani B, Brosnan CF, Ricciardi-Castagnoli P et al (2001) Developmental plasticity of CNS microglia. PNAS 98(11):6295–6300

Sarkar T, Patro N, Patro IK (2019) Cumulative multiple early life hits-a potent threat leading to neurological disorders. Brain Res Bull 147:58–68

Saxena K, Patro N, Patro I (2007) FK506 protects neurons following peripheral nerve injury via immunosuppression. Cell Mol Neurobiol 27(8):1049–1057

Sengupta P (2013) The laboratory rat: relating its age with humans Int J Prev Med 4(^): 624–630

Shaerzadeh F, Phan L, Miller D, Dacquel M, Hachmeister W, Hansen C et al (2020) Microglia senescence occurs in both substantia nigra and ventral tegmental area. Glia 68(11):2228–2245

Sharma SP, Patro IK, Patro N, James TJ (1988) 'Dark' type Purkinje cells and neuronal ageing. Proc Ind Acad Sci 97(5):449–453

Sharma A, Patro N, Patro IK (2016) Lipopolysaccharide induced apoptosis of astrocytes: therapeutic invention by minocycline. Cell Mol Neurobiol 36(4):577–592

Sheffield LG, Berman NE (1998) Microglial expression of MHC class II increases in normal aging of non-human primates. Neurobiol Aging 19(1):47–55

Sheng JG, Mrak RE, Griffin WS (1997) Neuritic plaque evolution in Alzheimer's disease is accompanied by transition of activated microglia from primed to enlarged to phagocytic forms. Acta Neuropathol 94(1):1–5

Shrivastava K, Gonzalez P, Acarin L (2012) The immune inhibitory complex CD200/CD200R is developmentally regulated in the mouse brain. J Comp Neurol 520(12):2657–2675

Sierra A, Gottfried-Blackmore AC, McEwen BS, Bulloch K (2007) Microglia derived from aging mice exhibit an altered inflammatory profile. Glia 55(4):412–424

Singh K, Patro N, Pradeepa M, Patro I (2017) Neonatal lipopolysaccharide infection causes demyelination and behavioral deficits in adult and senile rat brain. Ann Neurosci 24(3):146–154

Sinha S, Patro N, Patro IK (2019) Amelioration of neurobehavioral and cognitive abilities of F1 progeny following dietary supplementation with spirulina to protein malnourished mothers. Brain Behav Immun 85:69–87

Sinha S, Patro N, Tiwari PK, Patro IK (2020) Maternal spirulina supplementation during pregnancy and lactation partially prevents oxidative stress, glial activation and neuronal damage in protein malnourished F1 progeny. Neurochem Int 141:104877

Sosna J, Philipp S, Albay R, Reyes-Ruiz JM, Baglietto-Vargas D, LaFerla FM et al (2018) Early long-term administration of the CSF1R inhibitor PLX3397 ablates microglia and reduces accumulation of intraneuronal amyloid, neuritic plaque deposition and pre-fibrillar oligomers in 5XFAD mouse model of Alzheimer's disease. Mol Neurodegener 13(1):1–11

Spangenberg EE, Lee RJ, Najafi AR, Rice RA, Elmore MR, Blurton-Jones M et al (2016) Eliminating microglia in Alzheimer's mice prevents neuronal loss without modulating amyloid-β pathology. Brain 139(4):1265–1281

Sparkman NL, Johnson RW (2008) Neuroinflammation associated with aging sensitizes the brain to the effects of infection or stress. Neuroimmunomodulation 15(4–6):323–330

Stichel CC, Luebbert H (2007) Inflammatory processes in the aging mouse brain: participation of dendritic cells and T-cells. Neurobiol Aging 28(10):1507–1521

Stoll G, Jander S (1999) The role of microglia and macrophages in the pathophysiology of the CNS. Prog Neurobiol 58(3):233–247

Stopper L, Bălşeanu TA, Cătălin BO, Rogoveanu OC, Mogoantă LA, Scheller A (2018) Microglia morphology in the physiological and diseased brain-from fixed tissue to in vivo conditions. Romanian J Morphol Embryol 59(1):7–12

St-Pierre MK, Simoncicova E, Bogi E, Tremblay ME (2020) Shedding light on the dark side of the microglia. Am Soc Neurochem 12:1–10

Stratoulias V, Venero JL, Tremblay MÈ, Joseph B (2019) Microglial subtypes: diversity within the microglial community. EMBO J 38(17):101997

Streit WJ (2006) Microglial senescence: does the brain's immune system have an expiration date? Trends Neurosci 29(9):506–510
Streit WJ, Braak H, Xue QS, Bechmann I (2009) Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration I Alzheimer's disease. Acta Nauropathol 118(4):475–485
Streit WJ, Sammons NW, Kuhns AJ, Sparks DL (2004) Dystrophic microglia in the aging human brain. Glia 45(2):208–212
Streit WJ, Miller KR, Lopes KO, Njie E (2008) Microglial degeneration in the aging brain–bad news for neurons. Front Biosci 13:3423–3438
Streit WJ, Xue QS, Tischer J, Bechmann I (2014) Microglial pathology. Acta Neuropathol Commun 2(1):1–7
Streit WJ, Khoshbouei H, Bechmann I (2020) Dystrophic microglia in late-onset Alzheimer's disease. Glia 68(4):845–854
Swenson BL, Meyer CF, Bussian TJ, Baker DJ (2019) Senescence in aging and disorders of the central nervous system. Transl Mol Aging 3:17–25
Tambuyzer BR, Ponsaerts P, Nouwen EJ (2009) Microglia: gatekeepers of central nervous system immunology. J Leukoc Biol 85(3):352–370
Tay TL, Bechade C, D'Andrea I, St-Pierre MK, Henry MS, Roumier A, Tremblay ME (2017) Microglia gone rogue: impacts on psychiatric disorders across the life span. Front Mol Neurosci 10:421
Town T, Nikolic V, Tan J (2005) The microglial "activation" continuum: from innate to adaptive responses. J Neuroinflamm 2(1):24
Tremblay ME, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8:e1000527
Tremblay ME, Zhang I, Bisht K, Savage JC, Lecours C, Parent M (2016) Remodeling of lipid bodies by docosahexaenoic acid in activated microglial cells. J Neuroinflamm 3(1):1–8
Uranova NA, Vikhreva OV, Rakhmanova VI, Orlovskaya DD (2018) Ultrastructural pathology of oligodendrocytes adjacent to microglia in prefrontal white matter in schizophrenia. NP J Schizophr 4(1):1–10
Vida C, de Toda IM, Cruces J, Garrido A, Gonzalez-Sanchez M, De la Fuente M (2017) Role of macrophages in age-related oxidative stress and lipofuscin accumulation in mice. Redox Biol 12:423–437
Von Bernhardi R, Eugenín-von Bernhardi L, Eugenín J (2015) Microglial cell dysregulation in brain aging and neurodegeneration. Front Aging Neurosci 7:124
Walsh DM, Selkoe DJ (2004) Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron 44(1):181–193
Wang XJ, Zhang S, Yan ZQ, Zhao YX, Zhou HY, Wang Y et al (2011) Impaired CD200–CD200R-mediated microglia silencing enhances midbrain dopaminergic neurodegeneration: roles of aging, superoxide, NADPH oxidase, and p38 MAPK. Free Radic Biol Med 50(9):1094–1106
Wasserman JK, Yang H, Schlichter LC (2008) Glial responses, neuron death and lesion resolution after intracerebral hemorrhage in young vs. aged rats. Eur J Neurosci 28(7):1316–1328
Weinberg ED (2008) Iron out-of-balance: a risk factor for acute and chronic diseases. Hemoglobin 32(1–2):117–122
Wes PD, Holtman IR, Boddeke EW, Moller T, Eggen BJ (2016) Next generation transcriptomics and genomics elucidate biological complexity of microglia in health and disease. Glia 64(2): 197–213
Wong WT (2013) Microglial aging in the healthy CNS: phenotypes, drivers, and rejuvenation. Front Cell Neurosci 7:22
Wynne AM, Henry CJ, Huang Y, Cleland A, Godbout JP (2010) Protracted downregulation of CX3CR1 on microglia of aged mice after lipopolysaccharide challenge. Brain Behav Immun 24(7):1190–1201

Xie Z, Morgan TE, Rozovsky I, Finch CE (2003) Aging and glial responses to lipopolysaccharide in vitro: greater induction of IL-1 and IL-6, but smaller induction of neurotoxicity. Exp Neurol 182(1):135–141

Xu H, Chen M, Forrester JV (2009) Para-inflammation in the aging retina. Prog Retin Eye Res 28(5):348–368

Xu Y, Jin MZ, Yang ZY, Jin WL (2021) Microglia in neurodegenerative diseases. Neural Regen Res 16(2):270–280

Yang X, Ren H, Wood K, Li M, Qiu S, Shi FD (2018) Depletion of microglia augments the dopaminergic neurotoxicity of MPTP. FASEB J 32(6):3336–3345

Ye SM, Johnson RW (1999) Increased interleukin-6 expression by microglia from brain of aged mice. J Neuroimmunol 93(1–2):139–148

Youm YH, Grant RW, McCabe LR, Albarado DC, Nguyen KY, Ravussin A et al (2013) Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. Cell Metab 18(4):519–532

Zhang H, Sachdev PS, Wen W, Crawford JD, Brodaty H, Baune BT et al (2016) The relationship between inflammatory markers and voxel-based gray matter volumes in nondemented older adults. Neurobiol Aging 37:138–146

Zhang Q, Lu Y, Bian H, Guo L, Zhu H (2017) Activation of the α7 nicotinic receptor promotes lipopolysaccharide-induced conversion of M1 microglia to M2. Am J Transl Res 9(3):971–985

The Emerging Role of Satellite and Schwann Cells of the Peripheral Neuroglial System in Nerve Repair

Munmun Chattopadhyay

Abstract

The peripheral nervous system (PNS) has distinct neuroglial system that is different from the central nervous system glial population. The PNS consists of two types of glial cells, namely Schwann cells (SCs) and satellite glial cells (SGCs). Schwann cells are derived from the neural crest during development. The series of events in the formation of Schwann cells comprise of the formation of precursor Schwann cells, which then transform to immature Schwann cells. At this stage, these immature Schwann cells divide into two forms: myelinated and nonmyelinated (Remak) Schwann cells. Satellite glial cells are also derived from the neural crest and found in the PNS ganglia where they create an envelope around the sensory neurons as well as the sympathetic and parasympathetic ganglia neurons in the autonomic nervous system. The Schwann cells and the SGCs of the PNS take part actively in the regeneration process of the injured peripheral nerve to facilitate functional recovery. The Schwann cells are capable of modifying its role in response to injury by reorganizing the Remak (nonmyelin) and myelin phenotypes to form small and large myelinated axons which differ phenotypically and functionally from each other. To promote regeneration, Schwann cells and SGCs act simultaneously to respond to injury and create an environment to promote regeneration. This chapter will discuss the role of peripheral neuroglial system in peripheral nerve regeneration and the effectiveness and challenges of the nerve repair.

M. Chattopadhyay (✉)
Department of Molecular and Translational Medicine, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center El Paso, El Paso, TX, USA

Graduate School of Biomedical Sciences, Paul L. Foster School of MedicineTexas Tech University Health Sciences Center El Paso, El Paso, TX, USA
e-mail: munmun.chattopadhyay@ttuhsc.edu

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_21

Keywords

Schwann cells · Satellite glial cells · Peripheral nervous system · Regeneration · Nerve injury · Dorsal root ganglia

1 Introduction

Neuronal and glial cells are the fundamental cellular components of the nervous system. The central nervous system (CNS) consists of morphologically diverse glial cells as compared to the peripheral nervous system (PNS) (Jakel and Dimou 2017; Verkhratskiĭ and Butt 2013; von Bartheld et al. 2016). In the CNS, oligodendrocytes, microglia, and astrocytes are the main glial cell types, whereas Schwann cells and satellite glial cells are the key glial cells in the PNS. Specific glial cells are responsible for myelination of axons and coordinate with neuronal cell bodies and axonal projections for further function. Oligodendrocytes and Schwann cells (SCs) myelinate axons in the CNS and PNS respectively, and provide structural support. Myelination is an important component of nerves as it helps insulate the axons and participates in the propagation of nerve impulse.

The key functions of the glial cells of the CNS, i.e., astrocytes, microglia, and oligodendrocytes, are mainly to maintain chemical environment for signaling, myelination, and rate of nerve conduction and provide neuronal protection and integrity of synaptic function. Microglia are a form of macrophages of CNS, which mainly cleans and removes cellular remains from the injury sites, whereas Schwann cells and satellite glial cells are enfolded in the PNS (Jessen et al. 2015; Yang and Zhou 2019). Myelination of the axons in the PNS is an exclusive and important activity of the Schwann cells. Peripheral nerves also consist of nonmyelinating SCs. Both myelinating and nonmyelinating SCs play a significant role in the regeneration of peripheral nerve (Jessen and Mirsky 2016, 2019). Both myelinating and Remak (non-myelinating) Schwann cells provide metabolic and structural support to the axons. Satellite glial cells (SGCs) are found specifically in the sensory neurons of the PNS as well as in parasympathetic and sympathetic neurons of the autonomic nervous system (Hanani 2010; Hanani and Spray 2020). The SGCs ensheath the sensory neuronal cell bodies entirely and exhibit distinctive morphological features (Hanani and Verkhratsky 2021). The pathogenesis and function of satellite glial cells especially in the context of peripheral nerve repair are not well understood (George et al. 2018).

2 Myelinating and Nonmyelinating Schwann Cells

During development, the neural crest cells give rise to Schwann cells. There are two types of SCs that are developed from the neural crest (Woodhoo and Sommer 2008). In the developed PNS, SCs are classified into myelinating and nonmyelinating SCs (Fig. 21.1). There are two intermediate cell types, the precursor Schwann cell and the

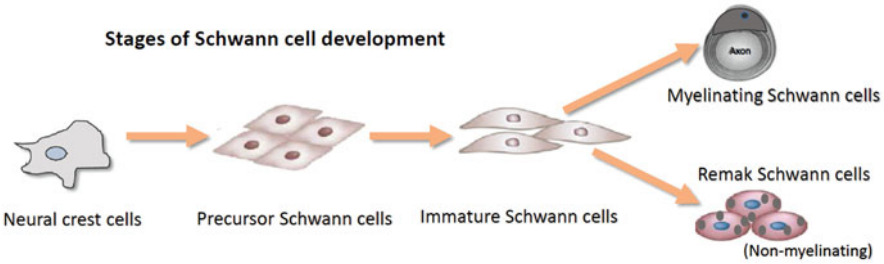

Fig. 21.1 Developmental Stages of Schwann cells: From Left: Neural crest cells transition to Schwann cell precursor lineage which then forms immature Schwann cells and finally these immature Schwann cells form myelinating and non-myelinating (Remak) Schwann cells

immature Schwann cell, which are derived from neural crest cells before forming the mature myelinating and nonmyelinating cell types. During SC differentiation from the immature Schwann cells, both anatomical and biochemical changes take place along with widespread alterations in gene expression and protein synthesis. In myelinating SCs, there is increased expression of peripheral myelin protein (PMP)-22, myelin basic protein (MBP), and peripheral myelin protein P0, whereas neural cell adhesion molecules, the neurotrophin receptor, p75, and glial fibrillary acidic protein (GFAP), are downregulated (Jessen and Mirsky 1999a, 1999b; Mirsky and Jessen 1999). Myelinating SCs ensheath myelin to large-diameter axons. Conversely, nonmyelinating SCs also known as Remak SCs (RSCs) wrap around the small caliber axons (<1 μm) to form Remak bundles (Harty and Monk 2017). Individual myelinating SC provides one layer of myelin sheath to a peripheral axon, and for highly myelinated axons, each resultant myelin sheath is composed by separate SCs, and a group of SCs is required for a long myelinated axon in the PNS (Kim et al. 2013). Therefore, SCs are the main source of myelination in peripheral nervous system and defined as the principal nonneuronal cells of PNS (Liu et al. 2019). SCs help myelinate axons, which also provide insulation to axons. Degree of myelination affects the rate of conduction velocity along the axon. On the other hand, nonmyelinating Schwann cells or Remak cells are critical for the maturation of the small caliber axons including the sensory axons of peripheral afferent neurons as well as the sympathetic and parasympathetic preganglionic fibers of the autonomic nervous system and provide support to these unmyelinated axons. Hence, these cells are even required for the maintenance and regeneration of the nerve following peripheral nerve injury (von Bartheld et al. 2016). Both SCs are considered as the major source for nutrients as well as metabolic and trophic support to the axons and play a crucial role in the development, maintenance, and function of the nerve. These two types of Schwann cells in the uninjured nerves exhibit a few morphological differences; myelinating SCs are two to three times longer and wider than the Remak cells, whereas Remak and myelinating SCs are maintained by similar cellular constituents; initially they are layered with basal lamina followed by endoneurium which comprises of fibroblasts, blood vessels, and few macrophages and are finally ensheathed by perineurium; altogether this assembly

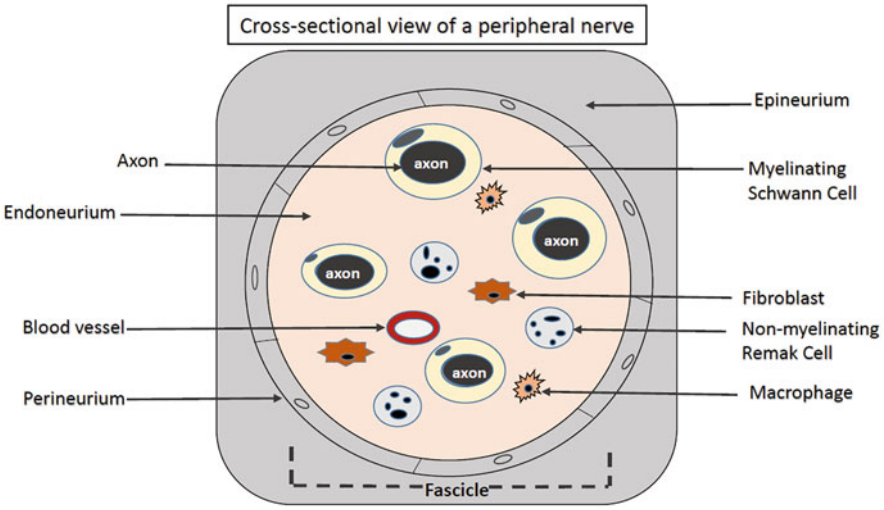

Fig. 21.2 Cross section of a peripheral nerve demonstrating the key cellular components. The nerve fascicle is surrounded by an epineurium. Each fascicle is separated by perineurium. The endoneurium consists of key cellular structures of the nerve consisting of a number of myelinating and nonmyelinating Schwann cells along with the connective tissue sections that consist of macrophages, fibroblasts, and blood vessels. The perineurium supports and protects the endoneurial components. Each epineurim may consist of a number of fascicles

is known as fascicle (Fig. 21.2). Most importantly, both of these Schwann cell types play distinct roles in the repair of the peripheral nerve injury.

3 Schwann Cells, Nerve Injury, and Repair

Peripheral nerve injury typically results from traumatic injury, recurrent overuse, drug-induced or from disease. Following injury, every so often the damaged axons fail to connect, retract from the target tissue, and eventually degenerate at the distal side of the injury. Therefore, the assessment of the depth of the peripheral nerve injuries through neurological evaluation is required which includes electromyography and nerve conduction studies, magnetic resonance imaging, computed tomography, as well as plain X-rays. Symptoms of the damaged nerve are frequently associated with changes in sensation causing numbness and tingling, burning, shooting, or sharp pain (Cruccu and Truini 2009). It is important to understand the nature of the injury that may also distinguish the basis of the pain between nerve-related and musculoskeletal origin. Pain may also be indicated as a process of normal nerve regeneration of the peripheral sensory fibers. Regeneration of the axons of the sensory neurons after nerve trauma facilitates a process for functional recovery. This creates a drastic alteration in the nerve-SC signaling milieu. Consequently, a large number of macrophages invade at the site of the damaged nerve and secrete a

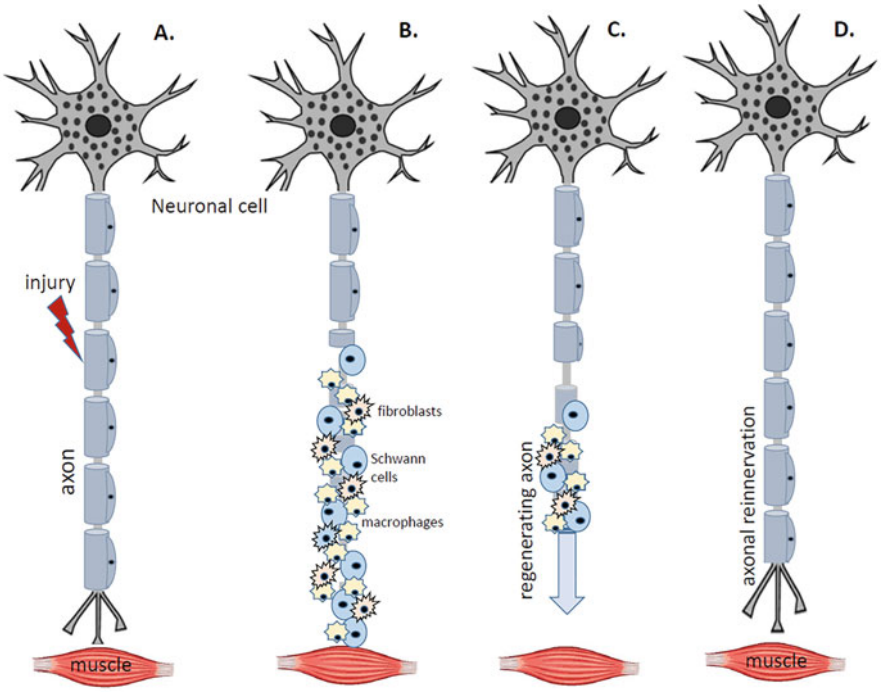

Fig. 21.3 Regeneration of the peripheral nerve: (**a**) injury to the nerve; (**b**) activation and recruitment of specific cells for restoration to the injury site by fibroblasts, SCs, and macrophages in response to degenerative signals; (**c**) active proliferating SCs forming a guiding path for the regenerating axon; and (**d**) effective axonal reinnervation toward the target tissue

number of bioactive elements (Jessen and Mirsky 2019). This leads to a significant alteration in the Schwann cell phenotype. Both nonmyelinating Remak cells and myelinating Schwann cells proceed toward repair mechanism which includes recruitment of macrophages and fibroblasts in this process to pursue regeneration (Murinson et al. 2005). The final phase of elongation and branching of the regenerating fibers is called Bungner bands; at that point, these fibers further proceed toward target innervation (Fig. 21.3).

To promote the healing process following injury, both neurons and SCs transform to specialized regenerating cell phenotypes to deal with injury and induce repairing process to implement Remak and myelinating SCs for restoration of the functional nerve (Gomez-Sanchez et al. 2017). These special SCs exist as needed. The process of alterations in cell phenotype with the aim of promoting nerve repair and homeostasis is defined as adaptive cellular reprogramming. The transformation of these cell phenotypes to restorative cells is regulated by the signaling mechanism which includes expression of transcriptional regulators such as Notch and c-Jun; these are activated by SCs of the injured nerves. The Raf/ERK pathway also contributes a crucial role in the repair process (Arthur-Farraj et al. 2012; Fontana et al. 2012;

Napoli et al. 2012). Remarkably, PNS neurons also activate a widespread gene expression system in response to axonal damage to expedite the axonal regeneration (Huebner and Strittmatter 2009). Therefore, the capability of SCs to transform into the repair phenotype after nerve damage enables the peripheral axon to regenerate after injury. Although this inherent process of sensory neurons to regulate nerve repair is now fairly known, the contribution of satellite glial cells (SGC) toward nerve regeneration is not fully understood (Feldman-Goriachnik and Hanani 2021). The most unique morphological feature of SGCs is that it entirely ensheaths the neuronal soma and these cells are found specifically in peripheral ganglia. Furthermore, a limited number of studies are performed to understand the differences between SGCs and SCs and their similarities with astrocytes (Hanani 2005).

Unlike CNS, neurons of PNS can regenerate and reinnervate following injury or trauma due to a supportive environment for axonal extension, survival, and growth. Several studies have shown that Schwann cells secrete various group of substances including neurotrophic factors like nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF); surface molecules such as integrin; extracellular matrix (ECM) proteins like laminin and fibronectin; and cell adhesion molecules such as N-CAM, which support or stimulate the axonal growth (Naidu 2009). Consequently, Schwann cell-mediated signals may have distinct effect on myelination in PNS compared to CNS after injury. Schwann cells from the distal part of the injured side exhibit increased expression of monocyte chemoattractant protein-1 (MCP-1) and leukemia inhibitory factor (LIF) to recruit and regulate macrophage responses and enhanced expression of pro-inflammatory cytokines including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-1α. Finally, SCs also initiate the myelin breakdown process after injury which further promotes the repair process in many ways (Jessen et al. 2015). During regeneration of axons following injury, Schwann cells invade the site of injury along with fibroblasts, macrophages, freshly developed blood vessels, and collagen fibers that create small bundles (Cattin et al. 2015). These axons may also create small loosely or densely arranged bundles typically devoid of fibroblasts. The thickness of these bundles depends on the amount of axons surrounding them. Consequently, the regenerating axon connects with the nerve bridge to reunite with the Schwann cells from the distal end of the transected nerve through an intricate signaling mechanism involving Sox2, ephrin-B/EphB2, and transforming growth factor (TGF)-β from regenerative axons, macrophages, and Schwann cells that also regulate the regenerative environment along with fibroblasts and blood vessels (Harty and Monk 2017). Furthermore, nerve injury can disrupt the normal myelination process and initiate the process of the formation of Büngner bands, and a greater percentage of these axons are associated within Remak bundles that could possibly cause hypersensitivity toward normal stimuli. The presence of these Remak bundles with larger fiber density could lead to pain phenotypes (Harty and Monk 2017).

Hence, one of the major limitations in nerve regeneration is the stability of the regenerating cell phenotype, which with time fails to sustain the expression of trophic factors that promote axonal restoration due to the alterations in the cell numbers and their signaling environment. It is important to understand that how

long the repair phenotype of the SCs is sustained particularly if the axon of the severed nerve is very long. In that case, the axon may take months to regenerate which may cause the loss of repairing ability of the Schwann cells in due course of time.

4 Neuroinflammation, Schwann Cells, and Satellite Glial Cells

Following peripheral nerve injury, the sensory neurons of the dorsal root ganglia (DRG) shift to regenerative phase to elicit regeneration and functional recovery of the peripheral nerve. The sensory neurons of DRG are ensheathed by SGCs to form an envelope, and each neuronal soma is completely wrapped by SGCs. The SGCs in the sensory ganglia of the PNS elicit significant changes following peripheral nerve damage. The hyperexcitability of the injured nerve instigates the release of inflammatory mediators and growth factors which may further cause changes in sensory neuron function that might contribute to pain. Nerve damage due to diabetes mellitus is one excellent example of the neuronal excitation which is mediated through SGC-derived release of cytokines, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β cytokines that further contribute to neuropathic pain (Goncalves et al. 2018). SGCs are modulated anatomically and functionally under pathological conditions, such as diabetes and chemotherapy-induced neuropathy, traumatic nerve injuries, and painful neuropathy (Warwick and Hanani 2013). SGCs express many common markers similar to glial cells. Nerve injury and inflammation increase the expression of GFAP (glial fibrillary acidic protein) in SGCs (Woodham et al. 1989). A number of studies reported that SGCs respond to the distal injury to the nerve with an increase in cell division and expression of sensory signals including increases in gap junction proteins GAP-43, ATP27, and increased release of pro-inflammatory cytokines such as TNFα, fractalkine, IL-6, and IL-1β, which may lead to increased neuronal excitability and firing (Dubovy et al. 2010; Souza et al. 2013). Sensory neurons are separated by SGCs and do not receive signals from the synapses, but rather SGCs contribute to cross depolarization. Peripheral inflammation or nerve injury contributes to increased electrical activity that leads upregulation to connexin-43 in SGCs (Takeda et al. 2007). Increased electrical stimulus due to injury causes release of vesicular ATP which further activates P2X7 purinergic receptors in the SGCs. This phenomenon initiates the release of neurotransmitters, and a bidirectional communication between neuronal soma and adjacent SGCs is established (X. Zhang et al. 2007). Nerve injury also attracts macrophages to the ganglia which are also responsible for release of cytokines that further leads to increased neuronal excitability. Cross talk between SGCs and macrophages orchestrate regeneration. Therefore, the sensory neurons and its satellite glial cells establish an environment that enhances neuron-SGC communication which could further be explored for SGC pharmacology to facilitate nerve repair (Pannese 2010).

5 Schwann Cells in Spinal Cord Injury and Repair

Injury to the spinal cord leads to an irreversible damage to the CNS neurons and surrounding environment. The evidence of the injury to the spinal cord encompasses with various characteristic indicators of prevalent cell death that could ultimately halt the axonal regeneration, which includes activation of an inflammatory milieu along with the development of glial scar in a growth inhibitory environment, which collectively could limit the axonal regrowth (Hutson and Di Giovanni 2019). This specific microenvironment affects the myelin production in the CNS by oligodendrocytes compared to myelination of the PNS by Schwann cells. In normal condition, single oligodendroglial cell could wrap several CNS axons, whereas a particular Schwann cell can encompass only single PNS axon. Postmitotic neurons in the CNS fail to regenerate after injury due to the inhibitory environment established by reactive glial cells along with astrocytic scars, chondroitin sulfate proteoglycans, and myelin debris, whereas in PNS, Schwann cells and the segments of myelin that they produce are regularly lost and replaced (Bradbury and Burnside 2019; Buss et al. 2004; Massey et al. 2008). Previous studies with CNS neurons demonstrated that after spinal cord injury or brain trauma, CNS neurons generally fail to regenerate, but these nerves tend to regrow from the damaged region of the axons when peripheral nerve segments are transplanted, occasionally with an ECM component (Cote et al. 2011; Houle et al. 2009; Tom et al. 2009). Recent evidences suggest that transplantation of Schwann cells is an encouraging therapeutic strategy for spinal cord repair. The implantation of SCs at the site of spinal cord injury has demonstrated an increase in axonal regeneration, with an enhanced sensorimotor function along with improved myelination of axons. Transplantation of SCs is the most often-applied procedure for nerve restoration in the injured spinal cord, and SCs are considered to be the most effective cell types for regeneration. Compared to peripheral nerve grafts, cultured SCs are preferred method of therapy for spinal cord trauma. These cells not only decrease the probabilities of any damage associated with nerve grafting but also create newly formed conduits of nerve tissue that get better adapted in the lesion site (Monje et al. 2021; Qu et al. 2021). All these studies suggest that Schwann cell establishes a milieu in the nerve that plays an essential role in supporting further axonal growth. This environment is further sustained by different cell types including immune cells such as endothelial cells, tissue-specific macrophages, and mesenchymal cells of both mesodermal and neural crest origin. Mesenchymal cells in the nerve actively participate in establishing connections to the gaps in the injured nerves (Feldman-Goriachnik and Hanani 2017; R. C. Zhang et al. 2021). Schwann cell-derived and peripheral nerve cell-mediated expression of growth factors forms a supportive environment that is considered to be beneficial to promote regeneration of axons in the CNS, as only Schwann cell transplantation is often inadequate. Besides the growth factors, extracellular matrix microenvironment in the peripheral axon is also greatly advantageous for axonal growth compared to the CNS, where distinct inhibitors of axonal regeneration dominate (Previtali et al. 2008). This supportive subcellular milieu that are partly derived from Schwann cells are composed of a basal lamina, ECM proteins, and cell adhesion molecules (Belin

et al. 2017; Chernousov and Carey 2000; Chernousov et al. 2008; Guseva et al. 2009).

6 Satellite Glial Cells in Nerve Repair and Pain

SGCs form an envelope of glial cells surrounding the neurons of the peripheral ganglia. Gap junction proteins connect these cells intercellularly around the neuronal soma (Fig. 21.4). The SGCs and the neuronal soma keep a space between them which consists of connective tissue components. The nonneuronal side of the SGCs establishes a blood-neuron barrier with a thin layer of basement membrane and endothelial basement membrane. The SGCs express a number of receptors and channels to maintain normal function of the neuron. Following an injury to the axon, these cells secrete neurotrophins to support the neuronal cell survival and maintain soma-SGC communication (Thippeswamy et al. 2005; Wetmore and Olson 1995). The physiological effect of SGCs on peripheral nerve restoration following injury has not been exploited widely. SGCs also share some common features with astrocytes; this includes expression of a number of proteins that were known for astrocytes, for example, glial fibrillary acidic protein (GFAP) and gap junction proteins, such as growth-associated protein 43 (GAP43) and connexin 43 (Avraham et al. 2020; Hanani et al. 2014; Ohara et al. 2008). Nevertheless, morphologically these cells are distinct with unique characteristics. SGCs are derived partly from neural crest cells and partly from neuroepithelial cells of the spinal cord (Sharma et al. 1995). The unique characteristics of SGCs do vary and it depends on their

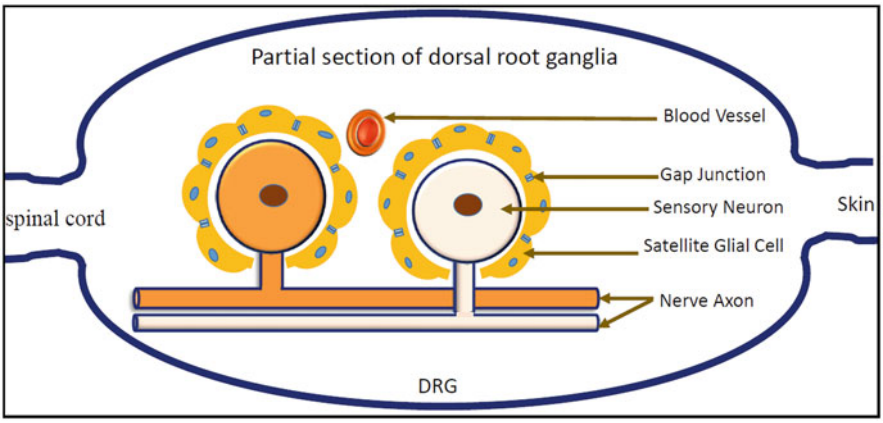

Fig. 21.4 Partial section of dorsal root ganglia (DRG) showing the sensory neurons with satellite glial cells (SGC) surrounding the neuronal soma. The pseudo-bipolar DRG neurons consists of sensory neurons that connect with peripheral tissues in the skin on one side and with the spinal cord dorsal horn on the other side. Detailed observation of the section of DRG shows that the neuronal soma of the sensory neurons is completely enveloped with SGCs (a few of which are outlined here) and gap junctions are present between the SGCs for communication

location; the SGCs found in DRG differ than those located in the trigeminal ganglia (TG) in terms of the morphology and distribution as well as sizes. Understanding the distinguishing characteristics of SGCS surrounding the TG and DRG neurons and its role as potential therapeutic targets under various physiological conditions (e.g., neuropathic and inflammatory) may advance the research on peripheral nerve regeneration. The advancement of investigation on Schwann cell in nerve repair and their role in downstream signaling to sensory neurons after nerve damage have been productive (Boerboom et al. 2017). The research on the validation of SCG role in peripheral nerve damage repair is ongoing presently (Jager et al. 2020). Recent studies have demonstrated that the synthesis of fatty acids by sensory neuron SGCs following nerve injury accelerates the regenerative process. A novel SGC mediated regenerative marker called Fabp7/BLBP (fatty acid binding protein 7/brain lipid-binding protein) has been identified during the nerve repair process. Changes or inhibition in fatty acid synthesis and peroxisome proliferator-activated receptor alpha (PPARα) signaling blocked or impaired nerve regeneration, whereas transcriptomic profiling of SCGs after nerve injury also verified that elimination of fatty acid synthase (Fasn) caused a deficiency in the axonal regeneration (Avraham et al. 2020). This investigation demonstrated that fatty acid synthesis in SGC after nerve injury is an important step in peripheral nerve repair. Nerve injury or inflammation often brings painful phenomenon, which can be present in acute phase of injury but can persist beyond that phase leading to a chronic stage. Therefore, the regenerative responses by SGCs after peripheral nerve injury can offer a new direction of investigation. Additional studies are required to understand the role of myelinating and nonmyelinating SCs in peripheral nerve injury. Identification of acute vs. chronic responses and the time course of the regeneration along with cell-cell interactions, formation of new satellite cells, alterations in function in SGCs of the damaged nerves, and changes in their role following the injury will be important to recognize how these interactions may impact on the total neuronal response.

In the recent past, studies have reported a specific type of peripheral glial cell with mesh-like network in the cutaneous tissues that are susceptible toward sensing noxious stimuli of thermal and mechanical origin. These specialized glial cells are uniquely connected to unmyelinated nerve fibers to deliver nociceptive signals to the nerve and are called nociceptive Schwann cells (Rinwa et al. 2021). These nociceptive Schwann cells are particularly important for maintenance of epidermal nociceptive fibers. Studies have shown that deletion of these nociceptive Schwann cells in rodents exhibits withdrawal of the nociceptive fibers leading to small fiber neuropathy and causing hypersensitive peripheral tissues. Additional studies are necessary to validate the role of these cutaneous SCs and to understand how these cells are associated to initiate pain-like behavior (Abdo et al. 2019). With potential options for the future treatment with SC transplantation for pain and peripheral nerve injury, there are opportunities to focus on SCs as therapeutic drug target for nerve regeneration which also justifies further studies to explore the etiology of small fiber neuropathy that may occur due to the damage to nociceptive Schwann cells.

Schwann cells are the major glial cell type in the peripheral nervous system and are responsible for the nerve regeneration by providing the supportive milieu toward

axonal myelination. Additionally, SGCs play an essential role in endogenous restorative features in peripheral nerve repair, along with axonal regeneration after an injury. Therefore, cell type-specific approaches for peripheral nerve repair will help us understand as how to enhance the repair-supportive functions. The advancement in the knowledge of diversified function of SGCs to maintain the nerve repair milieu will facilitate new perceptions in this field of research.

7 Conclusion

It is important to understand the intricacy of the alterations that may arise after peripheral nerve injury and the cross talk between Schwann cells and sensory neurons with other cell types including fibroblasts and macrophages besides the SGCs. The progress and accessibility of the high-end technological tools to study the transcriptomic profiles as well as morphological and physiological aspects of glial cells may further advance the field of SC plasticity. Translational research as well as testing and potential application of therapeutic strategies for glial cell-mediated therapy can influence the multifaceted actions of SCs. It is also critical to understand the interactions of SGCs with other types of cells including neurons and macrophages that mutually regulate responses to the peripheral nerve injury and inflammation. Strategies to conserve the functional aspect of SGCs to protect its role to rescue neural damage after injury or inflammation using pharmacological and non-pharmacological approaches are also important. The undesirable effect of SGCs on pain and its crucial role in nerve repair warrant further investigation. Emphasis on peripheral nerve regeneration via the delivery of SCs to the injury site seems realistic and achievable.

Besides SC transplantation and targeting SGC and SCs for drug target, there are more scopes such as 3D bioprints and possible application of induced pluripotent stem cells (iPSC) that would not only advance the investigation related to the intriguing science of neuroglial mechanisms in peripheral nerve repair but also will lead to safer and effective alternatives for patients suffering from nerve injury and pain.

It is also important to understand the role of the nonmyelinating Remak SCs compared to myelinating SCs in the context of peripheral nerve injury. Latest studies confirm that Remak SCs are involved in the developmental stages of the peripheral nerve as well as the axonal growth and regenerative process after damage. Therefore, it is important to expand our knowledge on the role of RSCs in the neuronal development and their promising contribution in the repair process of peripheral nerve damage.

References

Abdo H, Calvo-Enrique L, Lopez JM, Song J, Zhang MD, Usoskin D et al (2019) Specialized cutaneous Schwann cells initiate pain sensation. Science 365(6454):695–699. https://doi.org/10.1126/science.aax6452

Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A et al (2012) C-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. Neuron 75(4):633–647. https://doi.org/10.1016/j.neuron.2012.06.021

Avraham O, Deng PY, Jones S, Kuruvilla R, Semenkovich CF, Klyachko VA, Cavalli V (2020) Satellite glial cells promote regenerative growth in sensory neurons. Nat Commun 11(1):4891. https://doi.org/10.1038/s41467-020-18642-y

von Bartheld CS, Bahney J, Herculano-Houzel S (2016) The search for true numbers of neurons and glial cells in the human brain: a review of 150 years of cell counting. J Comp Neurol 524(18): 3865–3895. https://doi.org/10.1002/cne.24040

Belin S, Zuloaga KL, Poitelon Y (2017) Influence of mechanical stimuli on Schwann cell biology. Front Cell Neurosci 11:347. https://doi.org/10.3389/fncel.2017.00347

Boerboom A, Dion V, Chariot A, Franzen R (2017) Molecular mechanisms involved in Schwann cell plasticity. Front Mol Neurosci 10:38. https://doi.org/10.3389/fnmol.2017.00038

Bradbury EJ, Burnside ER (2019) Moving beyond the glial scar for spinal cord repair. Nat Commun 10(1):3879. https://doi.org/10.1038/s41467-019-11707-7

Buss A, Brook GA, Kakulas B, Martin D, Franzen R, Schoenen J et al (2004) Gradual loss of myelin and formation of an astrocytic scar during Wallerian degeneration in the human spinal cord. Brain 127(Pt 1):34–44. https://doi.org/10.1093/brain/awh001

Cattin AL, Burden JJ, Van Emmenis L, Mackenzie FE, Hoving JJ, Garcia Calavia N et al (2015) Macrophage-induced blood vessels guide Schwann cell-mediated regeneration of peripheral nerves. Cell 162(5):1127–1139. https://doi.org/10.1016/j.cell.2015.07.021

Chernousov MA, Carey DJ (2000) Schwann cell extracellular matrix molecules and their receptors. Histol Histopathol 15(2):593–601. https://doi.org/10.14670/HH-15.593

Chernousov MA, Yu WM, Chen ZL, Carey DJ, Strickland S (2008) Regulation of Schwann cell function by the extracellular matrix. Glia 56(14):1498–1507. https://doi.org/10.1002/glia.20740

Cote MP, Amin AA, Tom VJ, Houle JD (2011) Peripheral nerve grafts support regeneration after spinal cord injury. Neurotherapeutics 8(2):294–303. https://doi.org/10.1007/s13311-011-0024-6

Cruccu G, Truini A (2009) Tools for assessing neuropathic pain. PLoS Med 6(4):e1000045. https://doi.org/10.1371/journal.pmed.1000045

Dubovy P, Klusakova I, Svizenska I, Brazda V (2010) Satellite glial cells express IL-6 and corresponding signal-transducing receptors in the dorsal root ganglia of rat neuropathic pain model. Neuron Glia Biol 6(1):73–83. https://doi.org/10.1017/S1740925X10000074

Feldman-Goriachnik R, Hanani M (2017) The effects of endothelin-1 on satellite glial cells in peripheral ganglia. Neuropeptides 63:37–42. https://doi.org/10.1016/j.npep.2017.03.002

Feldman-Goriachnik R, Hanani M (2021) How do neurons in sensory ganglia communicate with satellite glial cells? Brain Res 1760:147384. https://doi.org/10.1016/j.brainres.2021.147384

Fontana X, Hristova M, Da Costa C, Patodia S, Thei L, Makwana M et al (2012) C-Jun in Schwann cells promotes axonal regeneration and motoneuron survival via paracrine signaling. J Cell Biol 198(1):127–141. https://doi.org/10.1083/jcb.201205025

George D, Ahrens P, Lambert S (2018) Satellite glial cells represent a population of developmentally arrested Schwann cells. Glia 66(7):1496–1506. https://doi.org/10.1002/glia.23320

Gomez-Sanchez JA, Pilch KS, van der Lans M, Fazal SV, Benito C, Wagstaff LJ et al (2017) After nerve injury, lineage tracing shows that myelin and Remak Schwann cells elongate extensively and branch to form repair Schwann cells, which shorten radically on Remyelination. J Neurosci 37(37):9086–9099. https://doi.org/10.1523/JNEUROSCI.1453-17.2017

Goncalves NP, Vaegter CB, Pallesen LT (2018) Peripheral glial cells in the development of diabetic neuropathy. Front Neurol 9:268. https://doi.org/10.3389/fneur.2018.00268

Guseva D, Angelov DN, Irintchev A, Schachner M (2009) Ablation of adhesion molecule L1 in mice favours Schwann cell proliferation and functional recovery after peripheral nerve injury. Brain 132(Pt 8):2180–2195. https://doi.org/10.1093/brain/awp160

Hanani M (2005) Satellite glial cells in sensory ganglia: from form to function. Brain Res Brain Res Rev 48(3):457–476. https://doi.org/10.1016/j.brainresrev.2004.09.001

Hanani M (2010) Satellite glial cells in sympathetic and parasympathetic ganglia: in search of function. Brain Res Rev 64(2):304–327. https://doi.org/10.1016/j.brainresrev.2010.04.009

Hanani M, Spray DC (2020) Emerging importance of satellite glia in nervous system function and dysfunction. Nat Rev Neurosci 21(9):485–498. https://doi.org/10.1038/s41583-020-0333-z

Hanani M, Verkhratsky A (2021) Satellite glial cells and astrocytes, a comparative review. Neurochem Res 46(10):2525–2537. https://doi.org/10.1007/s11064-021-03255-8

Hanani M, Blum E, Liu S, Peng L, Liang S (2014) Satellite glial cells in dorsal root ganglia are activated in streptozotocin-treated rodents. J Cell Mol Med 18(12):2367–2371. https://doi.org/10.1111/jcmm.12406

Harty BL, Monk KR (2017) Unwrapping the unappreciated: recent progress in Remak Schwann cell biology. Curr Opin Neurobiol 47:131–137. https://doi.org/10.1016/j.conb.2017.10.003

Houle JD, Amin A, Cote MP, Lemay M, Miller K, Sandrow H et al (2009) Combining peripheral nerve grafting and matrix modulation to repair the injured rat spinal cord. J Vis Exp (33). https://doi.org/10.3791/1324

Huebner EA, Strittmatter SM (2009) Axon regeneration in the peripheral and central nervous systems. Results Probl Cell Differ 48:339–351. https://doi.org/10.1007/400_2009_19

Hutson TH, Di Giovanni S (2019) The translational landscape in spinal cord injury: focus on neuroplasticity and regeneration. Nat Rev Neurol 15(12):732–745. https://doi.org/10.1038/s41582-019-0280-3

Jager SE, Pallesen LT, Richner M, Harley P, Hore Z, McMahon S et al (2020) Changes in the transcriptional fingerprint of satellite glial cells following peripheral nerve injury. Glia 68(7):1375–1395. https://doi.org/10.1002/glia.23785

Jakel S, Dimou L (2017) Glial cells and their function in the adult brain: a journey through the history of their ablation. Front Cell Neurosci 11:24. https://doi.org/10.3389/fncel.2017.00024

Jessen KR, Mirsky R (1999a) Developmental regulation in the Schwann cell lineage. Adv Exp Med Biol 468:3–12. https://doi.org/10.1007/978-1-4615-4685-6_1

Jessen KR, Mirsky R (1999b) Schwann cells and their precursors emerge as major regulators of nerve development. Trends Neurosci 22(9):402–410. https://doi.org/10.1016/s0166-2236(98)01391-5

Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. J Physiol 594(13):3521–3531. https://doi.org/10.1113/JP270874

Jessen KR, Mirsky R (2019) The success and failure of the Schwann cell response to nerve injury. Front Cell Neurosci 13:33. https://doi.org/10.3389/fncel.2019.00033

Jessen KR, Mirsky R, Lloyd AC (2015) Schwann cells: development and role in nerve repair. Cold Spring Harb Perspect Biol 7(7):a020487. https://doi.org/10.1101/cshperspect.a020487

Kim HA, Mindos T, Parkinson DB (2013) Plastic fantastic: Schwann cells and repair of the peripheral nervous system. Stem Cells Transl Med 2(8):553–557. https://doi.org/10.5966/sctm.2013-0011

Liu B, Xin W, Tan JR, Zhu RP, Li T, Wang D et al (2019) Myelin sheath structure and regeneration in peripheral nerve injury repair. Proc Natl Acad Sci U S A 116(44):22347–22352. https://doi.org/10.1073/pnas.1910292116

Massey JM, Amps J, Viapiano MS, Matthews RT, Wagoner MR, Whitaker CM et al (2008) Increased chondroitin sulfate proteoglycan expression in denervated brainstem targets following spinal cord injury creates a barrier to axonal regeneration overcome by chondroitinase ABC and neurotrophin-3. Exp Neurol 209(2):426–445. https://doi.org/10.1016/j.expneurol.2007.03.029

Mirsky R, Jessen KR (1999) The neurobiology of Schwann cells. Brain Pathol 9(2):293–311. https://doi.org/10.1111/j.1750-3639.1999.tb00228.x

Monje PV, Deng L, Xu XM (2021) Human Schwann cell transplantation for spinal cord injury: prospects and challenges in translational medicine. Front Cell Neurosci 15:690894. https://doi.org/10.3389/fncel.2021.690894

Murinson BB, Archer DR, Li Y, Griffin JW (2005) Degeneration of myelinated efferent fibers prompts mitosis in Remak Schwann cells of uninjured C-fiber afferents. J Neurosci 25(5): 1179–1187. https://doi.org/10.1523/JNEUROSCI.1372-04.2005

Naidu M (2009) The role of cells, neurotrophins, extracellular matrix and cell surface molecules in peripheral nerve regeneration. Malays J Med Sci 16(2):10–14

Napoli I, Noon LA, Ribeiro S, Kerai AP, Parrinello S, Rosenberg LH et al (2012) A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration in vivo. Neuron 73(4):729–742. https://doi.org/10.1016/j.neuron.2011.11.031

Ohara PT, Vit JP, Bhargava A, Jasmin L (2008) Evidence for a role of connexin 43 in trigeminal pain using RNA interference in vivo. J Neurophysiol 100(6):3064–3073. https://doi.org/10.1152/jn.90722.2008

Pannese E (2010) The structure of the perineuronal sheath of satellite glial cells (SGCs) in sensory ganglia. Neuron Glia Biol 6(1):3–10. https://doi.org/10.1017/S1740925X10000037

Previtali SC, Malaguti MC, Riva N, Scarlato M, Dacci P, Dina G et al (2008) The extracellular matrix affects axonal regeneration in peripheral neuropathies. Neurology 71(5):322–331. https://doi.org/10.1212/01.wnl.0000319736.43628.04

Qu WR, Zhu Z, Liu J, Song DB, Tian H, Chen BP et al (2021) Interaction between Schwann cells and other cells during repair of peripheral nerve injury. Neural Regen Res 16(1):93–98. https://doi.org/10.4103/1673-5374.286956

Rinwa P, Calvo-Enrique L, Zhang MD, Nyengaard JR, Karlsson P, Ernfors P (2021) Demise of nociceptive Schwann cells causes nerve retraction and pain hyperalgesia. Pain 162(6): 1816–1827. https://doi.org/10.1097/j.pain.0000000000002169

Sharma K, Korade Z, Frank E (1995) Late-migrating neuroepithelial cells from the spinal cord differentiate into sensory ganglion cells and melanocytes. Neuron 14(1):143–152. https://doi.org/10.1016/0896-6273(95)90248-1

Souza GR, Talbot J, Lotufo CM, Cunha FQ, Cunha TM, Ferreira SH (2013) Fractalkine mediates inflammatory pain through activation of satellite glial cells. Proc Natl Acad Sci U S A 110(27): 11193–11198. https://doi.org/10.1073/pnas.1307445110

Takeda M, Tanimoto T, Kadoi J, Nasu M, Takahashi M, Kitagawa J, Matsumoto S (2007) Enhanced excitability of nociceptive trigeminal ganglion neurons by satellite glial cytokine following peripheral inflammation. Pain 129(1–2):155–166. https://doi.org/10.1016/j.pain.2006.10.007

Thippeswamy T, McKay JS, Morris R, Quinn J, Wong LF, Murphy D (2005) Glial-mediated neuroprotection: evidence for the protective role of the NO-cGMP pathway via neuron-glial communication in the peripheral nervous system. Glia 49(2):197–210. https://doi.org/10.1002/glia.20105

Tom VJ, Sandrow-Feinberg HR, Miller K, Santi L, Connors T, Lemay MA, Houle JD (2009) Combining peripheral nerve grafts and chondroitinase promotes functional axonal regeneration in the chronically injured spinal cord. J Neurosci 29(47):14881–14890. https://doi.org/10.1523/JNEUROSCI.3641-09.2009

Verkhratskiĭ AN, Butt A (2013) Glial physiology and pathophysiology. Wiley-Blackwell, Chichester, West Sussex; Hoboken, NJ

Warwick RA, Hanani M (2013) The contribution of satellite glial cells to chemotherapy-induced neuropathic pain. Eur J Pain 17(4):571–580. https://doi.org/10.1002/j.1532-2149.2012.00219.x

Wetmore C, Olson L (1995) Neuronal and nonneuronal expression of neurotrophins and their receptors in sensory and sympathetic ganglia suggest new intercellular trophic interactions. J Comp Neurol 353(1):143–159. https://doi.org/10.1002/cne.903530113

Woodham P, Anderson PN, Nadim W, Turmaine M (1989) Satellite cells surrounding axotomised rat dorsal root ganglion cells increase expression of a GFAP-like protein. Neurosci Lett 98(1): 8–12. https://doi.org/10.1016/0304-3940(89)90364-9

Woodhoo A, Sommer L (2008) Development of the Schwann cell lineage: from the neural crest to the myelinated nerve. Glia 56(14):1481–1490. https://doi.org/10.1002/glia.20723

Yang QQ, Zhou JW (2019) Neuroinflammation in the central nervous system: symphony of glial cells. Glia 67(6):1017–1035. https://doi.org/10.1002/glia.23571

Zhang X, Chen Y, Wang C, Huang LY (2007) Neuronal somatic ATP release triggers neuron-satellite glial cell communication in dorsal root ganglia. Proc Natl Acad Sci U S A 104(23): 9864–9869. https://doi.org/10.1073/pnas.0611048104

Zhang RC, Du WQ, Zhang JY, Yu SX, Lu FZ, Ding HM et al (2021) Mesenchymal stem cell treatment for peripheral nerve injury: a narrative review. Neural Regen Res 16(11):2170–2176. https://doi.org/10.4103/1673-5374.310941

Insights in the Role of Glia in Mediating Brain Plasticity in Health and Disease

Vedangana Saini, Taranjeet Kaur, and Gurcharan Kaur

Abstract

Glial cells were originally known as simply the "glue" of the central nervous system but are now recognized to play a critical role in the development and function of the brain in both health and disease. Neurons and glial cells communicate with each other in a bidirectional manner at the synapses to regulate synaptic plasticity. Neuronal-glial interactions also initiate and maintain activation of membrane-bound proteins and subsequent intracellular downstream signaling events. Since glia and microglia are equal (and not silent) partners in creating neuroinflammatory milieu observed in diverse brain disorders, it is important to understand how these cells respond to internal and external cues to impact neuron functions under physiological and pathological conditions. Brain injury has a highly heterogeneous and multifactorial pathology, and the initial injury triggered by mechanical disruption often leads to the development of a secondary cascade of cellular/molecular responses. Astrocytes and microglia influence the local microenvironment of the injured tissue by their ability to secrete cytokines, chemokines, and growth factors and by undergoing profound morphological alterations to influence the extent of damage and repair following injury.

Keywords

Astrocytes · Microglia · Neuroinflammation · Plasticity · Injury

Vedangana Saini and Taranjeet Kaur contributed equally with all other contributors.

V. Saini · T. Kaur · G. Kaur (✉)
Department of Biotechnology, Guru Nanak Dev University, Amritsar, Punjab, India
e-mail: gurcharan.biotech@gndu.ac.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_22

This chapter will cover the following aspects:

- Glia, neuroinflammation, and neuronal plasticity.
- Glia and microglia in brain injury.

1 Glia, Neuroinflammation, and Neuronal Plasticity

Growing evidence suggests that glia play an indispensable role in major aspects of synapse development, function, and plasticity. The astrocytic and microglial processes are known to be associated with pre- and postsynaptic elements and affect synaptic functions, which have long been thought to be restricted to neurons. In a healthy brain, glial cells secrete soluble factors that induce synapse formation and modulate synaptic transmission and plasticity. Concurrently, synaptic signals influence glial cells by activating their cell surface receptors and modulating ion channels. It has also been established that peri-synaptic astrocytes and microglia are positioned to sense early disruptions in synaptic activity and potentially contribute to synaptic demise (Verkhratsky et al. 2015). It is well established that glia are affected in the nervous system pathologies; however, loss/dysfunction of synapses can occur long before the signs of neuropathology and cognitive impairment associated with the disease appear (Chung et al. 2015).

1.1 Role of Astrocytes in Synaptic Plasticity

Astrocytes are the most abundant cell type in the CNS, which are essential for regulating brain function. They are positioned in close physical association with synapses to sense and modulate synaptic activity. They interact with various cellular structures, including synapses and blood vessels, through their numerous processes. This close association enables them to function as first responders to various changes in synaptic activity during development and adulthood.

Astrocytes are known to participate actively in synaptic transmission and contribute to synaptic plasticity. The most important functions of astrocytes elucidated to date include uptake of the neurotransmitters, glutamate and gamma-aminobutyric acid, and potassium buffering during neuronal activity (Thrane et al. 2013). These functions are indispensable for maintaining synaptic activity. The dysregulation in these processes may lead to neuropathological conditions, including neuronal dysfunction and seizures. It has also been reported that astrocyte-secreted glutamine is critical for the sustained release of glutamate by neurons (Tani et al. 2014), which suggests that astrocytes play an active role in the production of the neurotransmitters used by the neurons. Recent research has led to the identification of several astrocytic factors that are capable of regulating synaptic plasticity. D-serine, secreted from astrocytes, has been reported to be involved in synaptic plasticity and

maintaining long-term potentiation by functioning as a co-agonist of NMDAR (Henneberger et al. 2010). ATP and glutamate have also been reported to be secreted by astrocytes through calcium-dependent vesicular exocytosis and contribute to synaptic plasticity (Halassa and Haydon 2010). A study by Han et al. (2013) suggests that human astrocytes may be better than rodent astrocytes in controlling the synaptic plasticity events underlying learning.

Further, in in vitro studies, the addition of astrocytes and astrocyte-conditioned media to purified culture of retinal ganglion cells has been shown to cause a significant increase in the number of functional excitatory synapses (Ullian et al. 2001). Chung et al. (2015) have reported that besides their role in synapse formation and function, astrocytes are also essential for synaptic maintenance. Synapses have been shown to immediately disappear when astrocytes or astrocyte-secreted signals are removed from cultured neurons (Chung et al. 2015). Thus, the formation of mature functional synapses requires multiple astrocyte-secreted signals, implicating the role of astrocytes in synapse formation and maintenance.

1.2 Role of Microglia in Synaptic Plasticity

Microglia are the resident macrophages of the CNS. The processes of microglia are known to interact with axonal boutons and dendritic spines. The direct contact between synaptic elements and microglia has been confirmed by electron microscopy (Tremblay et al. 2010). Each microglial cell can impact many synapses in response to external stimulus (Nimmerjahn et al. 2005). Microglia are also known to regulate maturation of synapses in both juvenile and mature brain. Interleukin (IL)-1β, a pro-inflammatory cytokine primarily expressed by microglia, has been implicated in the regulation of long-term potentiation in the hippocampus region (Williamson and Bilbo 2013; Zhang et al. 2014). Another pro-inflammatory cytokine, tumor necrosis factor α (TNFα), released by glia is required for synaptic scaling after a long-term reduction in activity (Stellwagen and Malenka 2006). It has been established that the expression of TNFα is highly enriched in microglia as compared to other brain cells; so, it may be inferred that microglia mediate the process of synaptic scaling. Thus, the ability of microglia to affect synapses through microglia-synapse interactions is crucial for proper CNS function.

1.3 Underlying Role of Glia in Synaptic Dysfunction in Neuropsychiatric Disorders

Dysfunction of synapses is the hallmark feature of majority of the neurological disorders. Synaptic dysfunction is often associated with the increased level of inflammation, which may further lead to cognitive impairments. Reactive gliosis and neuroinflammation are virtually prominent in every disorder of the central nervous system. Glia are regarded as passive responders to the neuronal damage, rather than the causal factor of synaptic dysfunction. Extensive research has led to

the realization of active glial signaling with neurons, which further influences synaptic transmission and plasticity through various contact-dependent and secreted mediators. Chronic neuroinflammation often leads to targeted disruption of synaptic plasticity, which further leads to compromised neuronal integrity. It is likely that astrocyte dysfunction contributes to the initiation and progression of many neurological disorders.

Microglia have been implicated as strong regulators of neurological function and cognition in physiological conditions (Singhal and Baune 2017). Although microglia can directly modulate cognition, it is noteworthy that they can also perform this role by secreting inflammatory mediators such as cytokines, whose role has been well established in influencing learning and memory functions of the brain. Disruption in the signaling pathways of pro-inflammatory cytokines, TNFα, IL-1β, and IL-6 has been shown to cause learning and memory impairments (Goshen et al. 2007; Hryniewicz et al. 2007; Baune et al. 2008; Camara et al. 2013), which suggests that these cytokines play an indispensable role in the physiological regulation of memory processes. The regulatory role of these cytokines has been reported to be dose-dependent, since the overexpression of TNFα and IL-1β has been shown to disrupt normal learning and memory functions in rodents (Fiore et al. 2000; Barrientos et al. 2002). Recent studies have shown that disruption in microglia activation may alter hippocampus-dependent neuronal plasticity affecting learning and memory performance in adulthood (Maggi et al. 2011; Rogers et al. 2011).

Cytokines and microglia may also impact cognition indirectly by modulating neurotrophic factors and the associated signaling pathways. Cytokines have been shown to modulate the activity and levels of brain-derived neurotrophic factor (BDNF) (Calabrese et al. 2014). Removal of BDNF from microglia has led to the revelation that microglia regulate memory by promoting synapse formation through BDNF signaling (Parkhurst et al. 2013). Further, it has been reported that inflammatory cytokines can influence the production of all the hormones produced along the hypothalamic-pituitary-adrenal (HPA) axis and modulate the function of glucocorticoid receptors at multiple stages from expression to translocation and associated signaling pathways (Pace and Miller 2009). Besides their effects on neurotrophic factors and HPA axis, inflammatory processes can influence activation of kynurenine pathway. Pro-inflammatory cytokines have been shown to induce hippocampal activation of the kynurenine-producing enzyme, indoleamine 2,3-dioxygenase (O'Connor et al. 2009), which participates in the regulation of memory and learning (Too et al. 2016). Thus, alterations in neurobiological processes regulating cognition are similar across various disorders. Below, we review the evidence which suggests that inflammatory processes in particular activated microglia and inflammatory cytokines play a major role in contributing to impaired cognitive performance associated with psychiatric disorders.

Major Depressive Disorder

In patients with major depressive disorder, elevated serum levels of TNFα, TNF receptor-type 1 (TNFR1), and TNF receptor-type 2 (TNFR2) have been shown to be negatively correlated with behavioral performance in learning, executive function,

attention, working, and declarative memories (Bobińska et al. 2017). Similarly, elevated levels of IL-6 and C-reactive protein (CRP) have been shown to be associated with impaired cognitive performance in the domains of verbal memory and psychomotor speed and of attention and executive functions, respectively (Chang et al. 2012; Goldsmith et al. 2016). It has also been shown that IL-6 and CRP levels can predict the symptoms of major depressive disorder at 12 years from the baseline detection. This suggests that inflammation contributes to the progression of major depressive disorder rather than to the later stages of the disease (Gimeno et al. 2009). This relationship may be unilateral since cognitive symptoms of depression at baseline were not found to be predictive of inflammatory status at 12 years' follow-up. Further, acute treatment with cyclooxygenase-2 inhibitor, celecoxib, has been shown to improve cognitive functions in an elderly depressed woman with recurrent major depressive disorder (Chen et al. 2010).

It has been suggested that kynurenine pathway may be involved in cognitive function impairment in patients with major depressive disorder. It has been implicated in influencing glutamatergic transmission in brain structures associated with cognitive processes (Fourrier et al. 2019). Alterations in glutamatergic synaptic plasticity have been linked to depression in animal models (Mahati et al. 2016). Additionally, inhibition of microglia activation has been shown to prevent impairment in both spatial memory and hippocampal long-term potentiation in a rodent model of depression. This effect has been attributed to GluR1 phosphorylation (Liu et al. 2015). BDNF, a neurotrophic factor, is also significantly associated with memory performance in rodents. The circulating levels of IL-6 are known to represent serum BDNF levels (Jehn et al. 2015); and the inhibition of TNFα has been shown to prevent stress-induced cognitive impairment and the associated reduction of hippocampal BDNF expression (Şahin et al. 2015).

Schizophrenia

Inflammation has also been extensively reported to be a potential player in the etiology and pathophysiology of schizophrenia. Cognitive impairment associated with schizophrenia has been correlated with increase in peripheral inflammation. A systemic review by Misiak et al. (2018) reported a positive association between circulating CRP levels and worse cognitive performance. Similarly, cognitive functions have been reported to be impaired in schizophrenic patients with elevated levels of circulating IL-6, TNFR1, and IL-1 receptor antagonist (Hope et al. 2015). The administration of an anti-inflammatory drug, risperidone, for 5 weeks has been shown to improve cognition in schizophrenic patients (Müller et al. 2005). Another study showed that minocycline added to a typical antipsychotic treatment has a beneficial effect on working memory and cognition. This suggests that inhibition of microglia activation in schizophrenic patients can decrease cognitive impairments (Levkovitz et al. 2010). This is in line with the microglia hypothesis of schizophrenia, which states that the neuropathology of this disease is closely associated with the increased activation of microglia (Monji et al. 2009; Laskaris et al. 2016). In animal models of schizophrenia, free radicals and inflammatory cytokines produced by activated microglia have been shown to cause decrease in neurogenesis, white matter

abnormalities, and neuronal degeneration, which may be the underlying factor in the pathophysiology of schizophrenia. It has also been suggested that increase in inflammation in schizophrenia may cause glutamatergic imbalance, leading to the dysfunction of dopaminergic system, which may in turn exacerbate glutamatergic transmission impairments, eventually leading to cognitive impairment (Müller 2008).

Bipolar Disorder

A few studies have reported association between inflammation and cognitive performance in patients with bipolar disorder. Circulating CRP levels have been shown to be negatively associated with memory and attention in patients with bipolar disorder (Dickerson et al. 2013). Similarly, elevated levels of IL-1 receptor antagonist and TNFα have been shown to be associated with worse memory performances (Hope et al. 2015). Elevated levels of soluble TNFRI have also been found to be associated with impaired declarative memory (Hoseth et al. 2016). A study evaluating the association between cerebrospinal fluid inflammatory markers and cognition in bipolar disorder patients reported a negative association between CSF concentration of the inflammatory biomarker YLK-40 and executive function in these patients (Rolstad et al. 2015). Besides increase in peripheral inflammation and cognitive impairments in bipolar disorder patients, increased microglial activation has been reported in the right hippocampus of bipolar disorder patients as compared to healthy controls (Haarman et al. 2014). Various underlying mechanisms have been suggested that participate in inflammation-associated cognitive impairments in bipolar disorder. A study suggests that pro-inflammatory cytokines such as TNFα impair white matter integrity in patients with bipolar disorder (Benedetti et al. 2016), which can be mediated by alterations in neurogenesis (Czéh and Lucassen 2007). Further, the activity of HPA axis may be influenced by cytokines, which may subsequently lead to impaired neuroplasticity. Indeed, HPA axis alterations have been associated with impaired cognition in patients with bipolar disorder. The insensitivity of glucocorticoid receptors has been reported in bipolar disorder (Fries et al. 2015), and mifepristone (glucocorticoid receptor antagonist) treatment for 1 week has been shown to improve spatial working memory performance in bipolar disorder patients (Watson et al. 2012). Additionally, it is noteworthy that peripheral BDNF level, which is regulated by inflammation, is an indicator for cognitive function in bipolar disorder patients. Moreover, the BDNF val66met polymorphism can be a risk factor for cognitive impairment in this disease (Bauer et al. 2014), which further reinforces the possible role of BDNF in mediating the effects of inflammation on cognition in bipolar disorder.

1.4 Studies from our Lab Exploring the Link Between Neuroinflammation and Synaptic Plasticity

Recently, our lab investigated the effect of pre-administration of water extract from leaves of *Withania somnifera* (ASH-WEX) and 50% ethanolic extract of *Tinospora*

cordifolia (TCE) on memory and cognitive impairment induced by acute sleep deprivation (Mishra et al. 2016; Kaur et al. 2017; Manchanda et al. 2017). In another study, the effect of administration of dry leaf powder of *W. somnifera* and stem powder of *T. cordifolia* along with high-fat diet (30% fat by weight) for the period of 12 weeks was explored (Kaur and Kaur 2017; Manchanda and Kaur 2017; Singh et al. 2021). The extracts of *W. somnifera* and *T. cordifolia*, well known for their psychotropic effects, were found to:

- Significantly improve memory impairment.
- Significantly reduce anxiety-like behavior.
- Reduce the expression of inflammatory markers such as TNFα, IL-1β, IL-6, GFAP, Iba1, OX-42, AP-1, and NF-κB.
- Reduce the stress-induced expression of PSA-NCAM and NCAM markers in the hippocampus and piriform cortex regions of the brain.

1.5 Remarks

The vulnerable cognitive impairments across many psychiatric disorders necessitate consideration since they affect not only the quality of life but also the treatment and recovery of patients. However, the underlying mechanisms for these deficits are not fully understood. These must be elucidated for better management of these disorders. The cognitive impairments across psychiatric conditions suggest shared mechanisms, potentially leading to their development. Neuroinflammation can be a shared underlying mechanism for the development of cognitive impairments in major depressive disorder, schizophrenia, and bipolar disorder. In fact, elevation in inflammatory processes, marked by the activation of microglia and increased levels of pro-inflammatory cytokines, can disrupt neurobiological mechanisms regulating cognitive processes. Though many studies have reported associations between inflammatory biomarkers, cognition-related biological mechanisms, and cognitive performance, causal evidence is still lacking.

2 Glia and Microglia in Brain Injury

Brain injury, defined as any insult to the CNS, has a multifactorial pathology. The initial injury triggered by mechanical disruption often leads to development of a secondary cascade of cellular/molecular responses. The glial cells of the CNS, astrocytes and microglia, are the key players involved in initiating the inflammatory cascade following injury. By their ability to secrete cytokines, chemokines, and growth factors and their ability to acquire new morphology, the astrocytes and microglia influence the local microenvironment of the injured tissue. Thus, they determine the extent of damage and repair following injury.

The role of glial cells in terms of damage versus repair has been considered ambiguous in the past (Pekny et al. 2014; Rust and Kaiser 2017). On one hand,

several authors have reported the pro-inflammatory and the detrimental aspects of the astrocytes and microglia toward axonal growth (Kitayama et al. 2011; Qian et al. 2019) following injury; on the other hand, studies have also shown the astrocytic and microglial response to injury to be beneficial for restricting damage and improving functional outcome (White et al. 2008; Mukaino et al. 2010). Over the years with the development in cellular and molecular techniques, it has become evident that the astrocytes and microglia are highly heterogeneous and their distinct subtypes are implicated in distinct cellular/molecular processes following injury (Anderson et al. 2014; Karve et al. 2016). However, very little is known about how and which subtypes are involved in the different functions. It is not established whether they are present in a continuum at the injury site or they have distinct topographical locations, which subtypes are involved in a particular type of injury such as spinal cord injury (SCI) or traumatic brain injury (TBI), or whether they get activated at different time points following injury. At this time, there are more questions than answers regarding the precise role of astrocytic and microglial subtypes following CNS injury. The following section has been designed to develop a comprehensive understanding of the astrocytic and microglial subtypes instrumental in the course of brain injury and the existing gaps in knowledge.

2.1 Astrocytes Following CNS Injury

Following trauma to the CNS, astrocytes undergo a series of structural, functional, cellular, molecular, as well as genetic changes collectively known as *astrogliosis* or *reactive astrocytosis* (Sofroniew and Vinters 2010). Astrogliosis occurs in all types of CNS injuries and involves complex interactions between astrocytes, neurons, other glial cells such as microglia, and the peripheral cells that enter the CNS through the bloodstream. This response is dependent on the severity of injury and is regulated specifically in different contexts via inter- and intracellular signaling molecules. Astrogliosis significantly alters astrocytic activities and plays an important role in determining the functional outcome in the long term following the insult. Three categories of astrogliosis have been reported in literature (Sofroniew and Vinters 2010) based on the severity of injury as well as the structural and molecular changes in the astrocytes: (a) mild/moderate, (b) severe diffuse, and (c) severe astrogliosis with glial scar formation (summarized in Table 22.1).

2.2 Astrocytic Subtypes in the Injury Response

Reactive astrocytosis was originally characterized by morphological changes in astrocytes such as hypertrophy and process remodeling as well as pronounced change in the expression of the intermediate filament, glial fibrillary acidic protein (GFAP) (Eng and Ghirnikar 1994). However, accumulating evidence from genetic studies over the course of years revealed that astrocytes have an ability to acquire several different types of morphologies and express several activation markers

Table 22.1 Categories of astrogliosis (adapted from Sofroniew and Vinters 2010)

	Mild/moderate astrogliosis	Severe diffuse astrogliosis	Severe astrogliosis with glial scar formation
Insult type	Mild injury, contusive injury, innate immune activation (diffuse)	Chronic neurodegeneration, diffuse injury and ischemia, infection	Inflammation following injury, stroke, infection, autoimmune diseases, neurodegenerative diseases
Astrocyte proliferation	No proliferation	Dispersed proliferation	Pronounced proliferation
Astrocyte topography	Distant to the injury site	Occur diffusely over substantial area	Astrocytic processes form compact scar borders that surround the injury site
Change in the gene expression of astrocytes	Pronounced change in gene expression	Pronounced change in gene expression	Pronounced change in gene expression
Morphological changes in astrocytes	Hypertrophy of cell body as well as the processes	Pronounced hypertrophy of cell body as well as the processes	Elongated cell bodies and processes
Change in individual astrocytic domain	No change	Some loss of the individual astrocytic domains	Astrocytic processes intertwine extensively

following the insult (Sofroniew 2014). Reactive astrocytes can upregulate GFAP to a similar extent following different stimuli and still can show different cell functions. Thus, a simple measure such as GFAP upregulation is not a good marker for astrocyte reactivity (Sofroniew 2014).

Reactive astrocytes depict heterogeneity at multiple levels (Sofroniew and Vinters 2010), and the astrocytic response to injury depends on their location with respect to the injury site, the activation state of astrocytes, and the signals they receive from their immediate environment as well as the maturation state of astrocytes (Sofroniew 2014). Several classifications of astrocytes following injury have recently been established. The following are the different types of astrocytes described in the literature that are implicated in the CNS injury response:

- **A1 versus A2 astrocytes:** Microarray profiling studies have classified reactive astrocytes into two types, namely, A1 and A2 astrocytes, depending on their mode of activation (Lin et al. 2004; Liddelow et al. 2017). According to this classification, A1 astrocytes are generated following inflammation or through the induction of inflammatory mediators such as TNFα, C1q, IL1, etc. A1 astrocytes have been demonstrated to be of pro-inflammatory nature and have been characterized to be neurotoxic to the growth of axons following injury (Liddelow et al. 2017). On the other hand, activation of astrocytes by ischemia results in A2 astrocytes (Lin et al. 2004). A2 astrocytes have been demonstrated to upregulate neurotrophic factors and thrombospondins which likely promote the survival and

growth of neurons and synapse repair, respectively. Hence, these astrocytes are considered as beneficial for axonal growth following injury (Liddelow and Barres 2017).

- **Scar forming versus hypertrophic stellate reactive astrocytes:** In another classification, reactive astrocytes are classified as scar forming and hypertrophic stellate astrocytes (Wanner et al. 2013). These astrocytes differ in their proximity to the injury site, morphology, and proliferative ability and are demonstrated to have a different source of origin. Scar forming astrocytes are proliferative astrocytes that are present in close proximity to the injury site. They mostly arise from the proliferation of the local astrocytes. These have an elongated morphology and have overlapping cell processes. These astrocytes have been demonstrated to have high expression of STAT3 (Wanner et al. 2013). Preventing astrocyte proliferation or STAT3 activation has been shown to promote tissue damage indicating that scar forming astrocytes are necessary for repair following CNS injury. Hypertrophic reactive astrocytes are stellate and non-proliferative. They are present distal to the injury site and derive directly from mature local astroglia whose processes overlap far less extensively or remain within the original territories (Wanner et al. 2013).
- **Spatial heterogeneity according to the embryonic sites or origin:** Cell-lineage fate mapping studies have shown that astrocytes are present in the mouse spinal cord in specific spatial domains or locations (Tsai et al. 2012). These localizations are in accordance with the embryonic sites of origin of the astrocytes.

2.3 Gaps in the Knowledge

In the literature, astrogliosis has been depicted to have a dual role (Sofroniew and Vinters 2010; Pekny et al. 2014; Sofroniew 2014). Reactive astrocytes have been shown to interact with both immune and inflammatory cells (Liddelow et al. 2017). Common changes that occur in astrocytes following injury include remodeling of molecular/cellular networks associated with cell morphology, growth, proliferation, and regulation of the cytokine production (Liddelow et al. 2017). Such changes are likely to derive astrocyte functions toward detrimental pro-inflammatory and beneficial trophic interactions with other cells. Whether there is a distinct spatial as well as temporal specialization among reactive astrocytes in this context is still unknown. Which astrocyte subtypes are expressed following specific CNS injuries such as SCI or TBI and where remain to be examined. It is unclear how the A1, A2, scar forming, or hypertrophic astrocytes interact with growing axons and impact their growth. This information is needed to appropriately target the astrocytic response following CNS injuries.

Further, it is still unclear if these astrocytic subtypes have clear distinctions or whether they share overlapping characteristics. Are the hypertrophic reactive astrocytes the same as A1 astrocytes, different astrocytic states, or different gradients of astrocytic activation is not known. Very little is known about the characteristics of astrocytes having different sources of origin. Not much is known about whether

reactive astrocytes derived from different precursor lineages might exhibit different characteristics or not. Such observations raise important questions about characterizing the signaling mechanisms or the gradients of cellular changes that create such heterogeneity.

2.4 Microglia Following CNS Injury

Microglia are dynamic cells that constantly survey the CNS environment for potential injury or insult (Kraft and Harry 2011). They are implicated in mounting an immune response after CNS injury. Similar to astrocytes, microglia also undergo several morphological and gene expression changes following injury which influence the damage versus repair effect (Loane and Byrnes 2010). The acute function of microglia following CNS injury is the removal of cellular/molecular debris (Kawabori and Yenari 2015). Generally, in the uninjured tissue, the resting or quiescent microglia have a ramified morphology (Glenn et al. 1992). Microglia at this stage have been shown to express receptors that recognize factors associated with tissue damage such as ATP, glutamate, growth factors, and cytokines (Jin and Yamashita 2016). Following CNS injury, the microglia acquire a hypertrophic or bushy morphology and upregulate the expression of the ionized calcium binding adaptor molecule 1 (Iba-1) and cluster of differentiation 68 (CD68) which promote active phagocytosis of the cellular debris (Jin and Yamashita 2016). This removal of damaged cells by microglia is very important for the restoration of the normal CNS functions as the factors released from the injured cells such as the Danger-associated molecular patterns (DAMPs) can promote considerable inflammation in the tissue (Roh and Sohn 2018). However, activated microglia, especially after chronic activation, have been shown to express noxious substances such as reactive oxygen species, reactive nitrogen species, excitatory neurotransmitters such as glutamate, as well as the pro-inflammatory cytokines which can lead to direct neurotoxic effects on growing axons (Kreutzberg 1996). The pro-inflammatory cytokines as well as the glutamate released by microglia also interfere with the normal functioning of the astrocytes (Takaki et al. 2012). Further, the microglial response following CNS injury has been shown to be context specific, i.e., dependent upon both the timing and the nature of the injury (Davalos et al. 2005).

2.5 Microglial Subtypes in the Injury Response

Similar to the astrocytes, microglial activation following CNS injury results in different phenotypes (Jin and Yamashita 2016). These phenotypes have been shown to correspond to both neurotoxic and neuroprotective priming states depending on the stage of the disease and the chronicity (Loane and Byrnes 2010; Kawabori and Yenari 2015; Jin and Yamashita 2016; Rust and Kaiser 2017). The following are the two types of microglia that have been shown to be implicated in the CNS injury response:

1. **Classically activated, M1 microglia:** M1 microglia are activated in situ by the pro-inflammatory cytokines such as IFN-γ, IL-1α, IL-6, and TNF-α. The M1 phenotype is implicated in secondary damage and scar formation following CNS injury (Hu et al. 2015). These microglia produce pro-inflammatory cytokines that are destructive to the growth of axons following injury. M1 microglia have been shown to be attracted more by the astrocyte enriched medium suggesting them to be more involved with the astrocytic interactions (Kirkley et al. 2017).
2. **Alternatively activated, M2 microglia:** The M2 phenotype of microglia is shown to be produced by activation with IL-4 and IL-13 (Jin and Yamashita 2016). M2 microglia are implicated in the phagocytosis after CNS injury. M2 microglia have been shown to produce scavenger receptors, growth factors such as TGF-β, and the anti-inflammatory cytokine IL-10 that are beneficial for axonal growth and repair following injury (Michelucci et al. 2009). M2 microglia are less inflammatory and more mobile than M1 microglia. These microglia have been shown to be attracted more by the neuron enriched medium (Matsui and Mori 2018).

2.6 Gaps in the Knowledge

The polarization of microglia following CNS injury is a highly dynamic process. Transcriptomic studies have revealed that microglia display a much broader transcriptional repertoire than M1 and M2 (Hickman et al. 2013; Xue et al. 2014). The polarization state can be dynamically altered depending on the severity of the insult, time following the injury, as well as the microenvironment. Many animal studies following TBI have shown a mixed expression of different markers associated with both M1 and M2 phenotypes (Kumar et al. 2016). Furthermore, several reports have suggested the microglial polarization to be a spectrum (Butovsky et al. 2005; Schwartz et al. 2006) rather than into two distinct groups (M1 or M2). Collectively, this suggests the *need to further characterize the polarization properties* of microglia specifically.

2.7 Remarks

The microenvironment following CNS injury implicates astrocytes and microglia in states that cannot be understood in the terms of typical immunological reactions. Whether astrocytes and microglia adopt a neurotoxic or neuroprotective role following injury depends on a number of factors such as the injury cause, severity and time course of injury, and the chemical signals present in the environment. The response depends on the heterogeneous subtypes involved as well as the bidirectional conversation between these subtypes. The languages of microglia and astrocytic subtypes will be the key to understand this complex system composed of cells of prodigious diversity as well as plasticity.

References

Anderson MA, Ao Y, Sofroniew MV (2014) Heterogeneity of reactive astrocytes. Neurosci Lett 565:23–29

Barrientos RM, Higgins EA, Sprunger DB, Watkins LR, Rudy JW, Maier SF (2002) Memory for context is impaired by a post context exposure injection of interleukin-1 beta into dorsal hippocampus. Behav Brain Res 134(1–2):291–298

Bauer IE, Pascoe MC, Wollenhaupt-Aguiar B, Kapczinski F, Soares JC (2014) Inflammatory mediators of cognitive impairment in bipolar disorder. J Psychiatr Res 56:18–27

Baune BT, Wiede F, Braun A, Golledge J, Arolt V, Koerner H (2008) Cognitive dysfunction in mice deficient for TNF-and its receptors. Am J Med Genet B Neuropsychiatr Genet 147(7): 1056–1064

Benedetti F, Poletti S, Hoogenboezem TA, Mazza E, Ambrée O, de Wit H, Wijkhuijs AJ, Locatelli C, Bollettini I, Colombo C, Arolt V (2016) Inflammatory cytokines influence measures of white matter integrity in bipolar disorder. J Affect Disord 202:1–9

Bobińska K, Gałecka E, Szemraj J, Gałecki P, Talarowska M (2017) Is there a link between TNF gene expression and cognitive deficits in depression? Acta Biochim Pol 64(1):65–73

Butovsky O, Talpalar AE, Ben-Yaakov K, Schwartz M (2005) Activation of microglia by aggregated β-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-γ and IL-4 render them protective. Mol Cell Neurosci 29(3):381–393

Calabrese F, Rossetti AC, Racagni G, Gass P, Riva MA, Molteni R (2014) Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. Front Cell Neurosci 8:430

Camara ML, Corrigan F, Jaehne EJ, Jawahar MC, Anscomb H, Koerner H, Baune BT (2013) TNF-α and its receptors modulate complex behaviours and neurotrophins in transgenic mice. Psychoneuroendocrinology 38(12):3102–3114

Chang HH, Lee IH, Gean PW, Lee SY, Chi MH, Yang YK, Lu RB, Chen PS (2012) Treatment response and cognitive impairment in major depression: association with C-reactive protein. Brain Behav Immun 26(1):90–95

Chen CY, Tzeng NS, Chen YC (2010) Maintenance therapy of celecoxib for major depression with mimicking neuropsychological dysfunction. Gen Hosp Psychiatry 32(6):647.e7–647.e9

Chung WS, Welsh CA, Barres BA, Stevens B (2015) Do glia drive synaptic and cognitive impairment in disease? Nat Neurosci 18(11):1539–1545

Czéh B, Lucassen PJ (2007) What causes the hippocampal volume decrease in depression? Eur Arch Psychiatry Clin Neurosci 257(5):250–260

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8(6): 752–758

Dickerson F, Stallings C, Origoni A, Vaughan C, Khushalani S, Yolken R (2013) Elevated C-reactive protein and cognitive deficits in individuals with bipolar disorder. J Affect Disord 150(2):456–459

Eng LF, Ghirnikar RS (1994) GFAP and astrogliosis. Brain Pathol 4(3):229–237

Fiore M, Angelucci F, Alleva E, Branchi I, Probert L, Aloe L (2000) Learning performances, brain NGF distribution and NPY levels in transgenic mice expressing TNF-alpha. Behav Brain Res 112(1–2):165–175

Fourrier C, Singhal G, Baune BT (2019) Neuroinflammation and cognition across psychiatric conditions. CNS Spectr 24(1):4–15

Fries GR, Vasconcelos-Moreno MP, Gubert C, Santos BT, Sartori J, Eisele B, Ferrari P, Fijtman A, Rüegg J, Gassen NC, Kapczinski F (2015) Hypothalamic-pituitary-adrenal axis dysfunction and illness progression in bipolar disorder. Int J Neuropsychopharmacol 18(1):pyu043

Gimeno D, Kivimäki M, Brunner EJ, Elovainio M, De Vogli R, Steptoe A, Kumari M, Lowe GD, Rumley A, Marmot MG, Ferrie JE (2009) Associations of C-reactive protein and interleukin-6

with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. Psychol Med 39(3):413–423
Glenn JA, Ward SA, Stone CR, Booth PL, Thomas WE (1992) Characterisation of ramified microglial cells: detailed morphology, morphological plasticity and proliferative capability. J Anat 180:109–118
Goldsmith DR, Haroon E, Woolwine BJ, Jung MY, Wommack EC, Harvey PD, Treadway MT, Felger JC, Miller AH (2016) Inflammatory markers are associated with decreased psychomotor speed in patients with major depressive disorder. Brain Behav Immun 56:281–288
Goshen I, Kreisel T, Ounallah-Saad H, Renbaum P, Zalzstein Y, Ben-Hur T, Levy-Lahad E, Yirmiya R (2007) A dual role for interleukin-1 in hippocampal-dependent memory processes. Psychoneuroendocrinology 32(8–10):1106–1115
Haarman BC, Riemersma-Van der Lek RF, de Groot JC, Ruhé HG, Klein HC, Zandstra TE, Burger H, Schoevers RA, de Vries EF, Drexhage HA, Nolen WA (2014) Neuroinflammation in bipolar disorder–a [11C]-(R)-PK11195 positron emission tomography study. Brain Behav Immun 40:219–225
Halassa MM, Haydon PG (2010) Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. Annu Rev Physiol 72:335–355
Han X, Chen M, Wang F, Windrem M, Wang S, Shanz S, Xu Q, Oberheim NA, Bekar L, Betstadt S, Silva AJ (2013) Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell Stem Cell 12(3):342–353
Henneberger C, Papouin T, Oliet SH, Rusakov DA (2010) Long-term potentiation depends on release of D-serine from astrocytes. Nature 463(7278):232–236
Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang LC, Means TK, El Khoury J (2013) The microglial sensome revealed by direct RNA sequencing. Nat Neurosci 16(12):1896–1905
Hope S, Hoseth E, Dieset I, Mørch RH, Aas M, Aukrust P, Djurovic S, Melle I, Ueland T, Agartz I, Ueland T (2015) Inflammatory markers are associated with general cognitive abilities in schizophrenia and bipolar disorder patients and healthy controls. Schizophr Res 165(2–3): 188–194
Hoseth EZ, Westlye LT, Hope S, Dieset I, Aukrust P, Melle I, Haukvik UK, Agartz I, Ueland T, Ueland T, Andreassen OA (2016) Association between cytokine levels, verbal memory and hippocampus volume in psychotic disorders and healthy controls. Acta Psychiatr Scand 133(1): 53–62
Hryniewicz A, Bialuk I, Kamiński KA, Winnicka MM (2007) Impairment of recognition memory in interleukin-6 knock-out mice. Eur J Pharmacol 577(1–3):219–220
Hu X, Leak RK, Shi Y, Suenaga J, Gao Y, Zheng P, Chen J (2015) Microglial and macrophage polarization—new prospects for brain repair. Nat Rev Neurol 11(1):56–64
Jehn CF, Becker B, Flath B, Nogai H, Vuong L, Schmid P, Lüftner D (2015) Neurocognitive function, brain-derived neurotrophic factor (BDNF) and IL-6 levels in cancer patients with depression. J Neuroimmunol 287:88–92
Jin X, Yamashita T (2016) Microglia in central nervous system repair after injury. J Biochem 159(5):491–496
Karve IP, Taylor JM, Crack PJ (2016) The contribution of astrocytes and microglia to traumatic brain injury. Br J Pharmacol 173(4):692–702
Kaur T, Kaur G (2017) *Withania somnifera* as a potential candidate to ameliorate high fat diet-induced anxiety and neuroinflammation. J Neuroinflammation 14(1):201
Kaur T, Singh H, Mishra R, Manchanda S, Gupta M, Saini V, Sharma A, Kaur G (2017) *Withania somnifera* as a potential anxiolytic and immunomodulatory agent in acute sleep deprived female Wistar rats. Mol Cell Biochem 427(1–2):91–101
Kawabori M, Yenari MA (2015) The role of the microglia in acute CNS injury. Metab Brain Dis 30(2):381–392
Kirkley KS, Popichak KA, Afzali MF, Legare ME, Tjalkens RB (2017) Microglia amplify inflammatory activation of astrocytes in manganese neurotoxicity. J Neuroinflammation 14(1):99

Kitayama M, Ueno M, Itakura T, Yamashita T (2011) Activated microglia inhibit axonal growth through RGMa. PLoS One 6(9):e25234

Kraft AD, Harry GJ (2011) Features of microglia and neuroinflammation relevant to environmental exposure and neurotoxicity. Int J Environ Res Public Health 8(7):2980–3018

Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19(8):312–318

Kumar A, Alvarez-Croda DM, Stoica BA, Faden AI, Loane DJ (2016) Microglial/macrophage polarization dynamics following traumatic brain injury. J Neurotrauma 33(19):1732–1750

Laskaris LE, Di Biase MA, Everall I, Chana G, Christopoulos A, Skafidas E, Cropley VL, Pantelis C (2016) Microglial activation and progressive brain changes in schizophrenia. Br J Pharmacol 173(4):666–680

Levkovitz Y, Mendlovich S, Riwkes S, Braw Y, Levkovitch-Verbin H, Gal G, Fennig S, Treves I, Kron S (2010) A double-blind, randomized study of minocycline for the treatment of negative and cognitive symptoms in early-phase schizophrenia. J Clin Psychiatry 71(2):138–149

Liddelow SA, Barres BA (2017) Reactive astrocytes: production, function, and therapeutic potential. Immunity 46(6):957–967

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung WS, Peterson TC, Wilton DK (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541(7638):481–487

Lin TN, Wang Q, Simonyi A, Chen JJ, Cheung WM, He Y, Xu J, Sun AY, Hsu CY, Sun GY (2004) Induction of secretory phospholipase A2 in reactive astrocytes in response to transient focal cerebral ischemia in the rat brain. J Neurochem 90(3):637–645

Liu M, Li J, Dai P, Zhao F, Zheng G, Jing J, Wang J, Luo W, Chen J (2015) Microglia activation regulates GluR1 phosphorylation in chronic unpredictable stress-induced cognitive dysfunction. Stress 18(1):96–106

Loane DJ, Byrnes KR (2010) Role of microglia in neurotrauma. Neurotherapeutics 7(4):366–377

Maggi L, Scianni M, Branchi I, D'Andrea I, Lauro C, Limatola C (2011) CX3CR1 deficiency alters hippocampal-dependent plasticity phenomena blunting the effects of enriched environment. Front Cell Neurosci 5:22

Mahati K, Bhagya V, Christofer T, Sneha A, Rao BSS (2016) Enriched environment ameliorates depression-induced cognitive deficits and restores abnormal hippocampal synaptic plasticity. Neurobiol Learn Mem 134:379–391

Manchanda S, Kaur G (2017) *Withania somnifera* leaf alleviates cognitive dysfunction by enhancing hippocampal plasticity in high fat diet induced obesity model. BMC Complement Altern Med 17(1):136

Manchanda S, Mishra R, Singh R, Kaur T, Kaur G (2017) Aqueous leaf extract of *Withania somnifera* as a potential neuroprotective agent in sleep-deprived rats: a mechanistic study. Mol Neurobiol 54(4):3050–3061

Matsui TK, Mori E (2018) Microglia support neural stem cell maintenance and growth. Biochem Biophys Res Commun 503(3):1880–1884

Michelucci A, Heurtaux T, Grandbarbe L, Morga E, Heuschling P (2009) Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: effects of oligomeric and fibrillar amyloid-β. J Neuroimmunol 210(1–2):3–12

Mishra R, Manchanda S, Gupta M, Kaur T, Saini V, Sharma A, Kaur G (2016) *Tinospora cordifolia* ameliorates anxiety-like behavior and improves cognitive functions in acute sleep deprived rats. Sci Rep 6:25,564

Misiak B, Stańczykiewicz B, Kotowicz K, Rybakowski JK, Samochowiec J, Frydecka D (2018) Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: a systematic review. Schizophr Res 192:16–29

Monji A, Kato T, Kanba S (2009) Cytokines and schizophrenia: microglia hypothesis of schizophrenia. Psychiatry Clin Neurosci 63(3):257–265

Mukaino M, Nakamura M, Yamada O, Okada S, Morikawa S, Renault-Mihara F, Iwanami A, Ikegami T, Ohsugi Y, Tsuji O, Katoh H (2010) Anti-IL-6-receptor antibody promotes repair of spinal cord injury by inducing microglia-dominant inflammation. Exp Neurol 224(2):403–414

Müller N (2008) Inflammation and the glutamate system in schizophrenia: implications for therapeutic targets and drug development. Expert Opin Ther Targets 12(12):1497–1507

Müller N, Riedel M, Schwarz MJ, Engel RR (2005) Clinical effects of COX–2 inhibitors on cognition in schizophrenia. Eur Arch Psychiatry Clin Neurosci 255(2):149–151

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318

O'Connor JC, André C, Wang Y, Lawson MA, Szegedi SS, Lestage J, Castanon N, Kelley KW, Dantzer R (2009) Interferon-γ and tumor necrosis factor-α mediate the upregulation of indoleamine 2, 3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guérin. J Neurosci 29(13):4200–4209

Pace TW, Miller AH (2009) Cytokines and glucocorticoid receptor signaling: relevance to major depression. Ann N Y Acad Sci 1179:86–105

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR III, Lafaille JJ, Hempstead BL, Littman DR, Gan WB (2013) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155(7):1596–1609

Pekny M, Wilhelmsson U, Pekna M (2014) The dual role of astrocyte activation and reactive gliosis. Neurosci Lett 565:30–38

Qian D, Li L, Rong Y, Liu W, Wang Q, Zhou Z, Gu C, Huang Y, Zhao X, Chen J, Fan J (2019) Blocking notch signal pathway suppresses the activation of neurotoxic A1 astrocytes after spinal cord injury. Cell Cycle 18(21):3010–3029

Rogers JT, Morganti JM, Bachstetter AD, Hudson CE, Peters MM, Grimmig BA, Weeber EJ, Bickford PC, Gemma C (2011) CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J Neurosci 31(45):16241–16250

Roh JS, Sohn DH (2018) Damage-associated molecular patterns in inflammatory diseases. Immune Netw 18(4):e27

Rolstad S, Jakobsson J, Sellgren C, Isgren A, Ekman CJ, Bjerke M, Blennow K, Zetterberg H, Pålsson E, Landén M (2015) CSF neuroinflammatory biomarkers in bipolar disorder are associated with cognitive impairment. Eur Neuropsychopharmacol 25(8):1091–1098

Rust R, Kaiser J (2017) Insights into the dual role of inflammation after spinal cord injury. J Neurosci 37(18):4658–4660

Şahin TD, Karson A, Balcı F, Yazır Y, Bayramgürler D, Utkan T (2015) TNF-alpha inhibition prevents cognitive decline and maintains hippocampal BDNF levels in the unpredictable chronic mild stress rat model of depression. Behav Brain Res 292:233–240

Schwartz M, Butovsky O, Brück W, Hanisch UK (2006) Microglial phenotype: is the commitment reversible? Trends Neurosci 29(2):68–74

Singh H, Bajaj P, Kalotra S, Bhandari A, Kaur T, Singh AP, Kaur G (2021) *Tinospora cordifolia* ameliorates brain functions impairments associated with high fat diet induced obesity. Neurochem Int 143:104937

Singhal G, Baune BT (2017) Microglia: an interface between the loss of neuroplasticity and depression. Front Cell Neurosci 11:270

Sofroniew MV (2014) Astrogliosis. Cold Spring Harb Perspect Biol 7(2):a020420

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119(1): 7–35

Stellwagen D, Malenka RC (2006) Synaptic scaling mediated by glial TNF-α. Nature 440(7087): 1054–1059

Takaki J, Fujimori K, Miura M, Suzuki T, Sekino Y, Sato K (2012) L-glutamate released from activated microglia downregulates astrocytic L-glutamate transporter expression in neuroinflammation: the 'collusion'hypothesis for increased extracellular L-glutamate concentration in neuroinflammation. J Neuroinflammation 9:275

Tani H, Dulla CG, Farzampour Z, Taylor-Weiner A, Huguenard JR, Reimer RJ (2014) A local glutamate-glutamine cycle sustains synaptic excitatory transmitter release. Neuron 81(4): 888–900

Thrane VR, Thrane AS, Wang F, Cotrina ML, Smith NA, Chen M, Xu Q, Kang N, Fujita T, Nagelhus EA, Nedergaard M (2013) Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. Nat Med 19(12):1643–1648

Too LK, Li KM, Suarna C, Maghzal GJ, Stocker R, McGregor IS, Hunt NH (2016) Deletion of TDO2, IDO-1 and IDO-2 differentially affects mouse behavior and cognitive function. Behav Brain Res 312:102–117

Tremblay MÈ, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8(11):e1000527

Tsai HH, Li H, Fuentealba LC, Molofsky AV, Taveira-Marques R, Zhuang H, Tenney A, Murnen AT, Fancy SP, Merkle F, Kessaris N (2012) Regional astrocyte allocation regulates CNS synaptogenesis and repair. Science 337(6092):358–362

Ullian EM, Sapperstein SK, Christopherson KS, Barres BA (2001) Control of synapse number by glia. Science 291(5504):657–661

Verkhratsky A, Nedergaard M, Hertz L (2015) Why are astrocytes important? Neurochem Res 40(2):389–401

Wanner IB, Anderson MA, Song B, Levine J, Fernandez A, Gray-Thompson Z, Ao Y, Sofroniew MV (2013) Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. J Neurosci 33(31):12870–12886

Watson S, Gallagher P, Porter RJ, Smith MS, Herron LJ, Bulmer S, Young AH, Ferrier IN, North-East Mood Disorders Clinical Research Group (2012) A randomized trial to examine the effect of mifepristone on neuropsychological performance and mood in patients with bipolar depression. Biol Psychiatry 72(11):943–949

White RE, Yin FQ, Jakeman LB (2008) TGF-α increases astrocyte invasion and promotes axonal growth into the lesion following spinal cord injury in mice. Exp Neurol 214(1):10–24

Williamson LL, Bilbo SD (2013) Chemokines and the hippocampus: a new perspective on hippocampal plasticity and vulnerability. Brain Behav Immun 30:186–194

Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, De Nardo D, Gohel TD, Emde M, Schmidleithner L, Ganesan H (2014) Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity 40(2):274–288

Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34(36): 11929–11947

The Glial Perspective of Energy Homeostasis, Neuroinflammation, and Neuro-nutraceuticals

Shrstha Sinha, Nisha Patro, and Ishan Patro

Abstract

Millions of people worldwide suffer from various neurodegenerative conditions that impact behavioral and cognitive performance. Any disturbance in neuron-glia and glia-glia interactions along with associated signaling cascades is the governing factor in the pathophysiology of spectrum of neurological disorders. This chapter highlights the role of glial cells in regulating energy homeostasis (astrocytes, tanycytes, and microglia) and inflammatory response and activity of naturally occurring immunomodulators which are necessary for overall body and brain health. The results obtained from various research studies on curcumin, blueberries, *Withania somnifera*, *Tinospora cordifolia*, *Bacopa monnieri*, *Ganoderma lucidum*, *Allium sativum*, and *Spirulina* are summarized here that might be beneficial for promoting research on brain health and cognitive performance in neurodegenerative conditions. Despite extensive research on the role of nutraceuticals in positively maintaining brain health, the glial involvement underlying these benefits remains only partly understood. Nevertheless, future studies are warranted in the field to address glioprotective potential of nutraceutical against neurodegenerative disorders.

Keywords

Energy homeostasis · Immunomodulators · Neuroinflammation · Neuro-nutraceuticals

S. Sinha · N. Patro · I. Patro (✉)
School of Studies in Neuroscience, Jiwaji University, Gwalior, Madhya Pradesh, India
e-mail: ikpatro@jiwaji.edu

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_23

1 Introduction

Exposure to various stressors (neurotoxin insult, malnutrition, trauma, injury) during early and/or later life negatively affects CNS microenvironment and enhances the risk of neurological disorders by manyfold. Both neuronal and nonneuronal cells function as obligatory partners in regulating metabolic changes in the brain. Glial cells are the most abundant cells in the nervous system and have been implicated in both physiology and pathology of nervous system. More recently, we have noted maternal protein deprivation to be associated with compromised gliogenesis (Naik et al. 2017; Patro et al. 2019) which further contributes to behavioral and cognitive deficits in F1 progeny (Naik et al. 2015; Sinha et al. 2020a). Current research reports suggest that astrocytes, microglia, and tanycytes actively participate in physiological and pathophysiological procedures of energy balance by regulating neuronal circuitry (Argente-Arizon et al. 2016). Disturbed neuron-glia and glia-glia cross talk constitute a key mechanism involved in neuroinflammation and metabolic dysfunctions (Reviewed in details by Jana et al. 2016).

The majority of neurodegenerative disorders share common pathways like oxidative stress, inflammation, and mitochondrial dysfunction. The existence of multiple pathways simultaneously affects the outcome of therapeutic interventions in neurodegenerative conditions. Thus, numerous target-based therapies including second messengers, neurotransmitter modulators, stem cells, neural transplantation, unfolded protein response, receptor agonist/antagonists, and hormone replacement therapies have been developed for the treatment of neurodegenerative disorders (Young 2009; Dantuma et al. 2010; Dye et al. 2012; Moraes 2015). Unfortunately, these treatment options also cause severe long-term adverse effects (Morrish 2012; Roch-Torreilles et al. 2000). Thus, there is an urgent need to recognize safer and economic alternative options for the treatment of neurological disorders.

Nutraceuticals have gained significant momentum and are known to target multiple pathways with minimum or no adverse effects (Dadhania et al. 2016). The term nutraceutical is derived from nutrition and pharmaceutical and refers to any food or food part with nutritional and medicinal properties. Generally, nutraceutical-based research follows traditional medicine practices. For example, Charaka Samhita, Susrutha Samhita, and Ashtangahrdayam are the ancient texts of Indian Ayurveda system (Menon and Spudich 2010). Similarly, Chinese and Japanese traditional medicines have now become a part of many pharmaceutical preparations. All the nutraceuticals which improve brain health and aid in behavioral and cognitive improvements are considered as "neuro-nutraceuticals" (Williams et al. 2016). Despite significant studies on the role of nutraceuticals in human health, they are largely considered as a source of antioxidants only. Neuro-nutraceuticals are generally believed to exert immunomodulatory effects in brain cells by targeting a number of signaling cascades along with their anti-inflammatory, antioxidant, and antiapoptotic properties.

Recent work from our lab proposed the beneficial impact of maternal *Spirulina* supplementation against protein malnutrition-associated cognitive deficits (Sinha et al. 2020a), thereby deciphering the effectiveness of *Spirulina* as a functional

food having both nutritional and medicinal properties (Sinha et al. 2018, 2020b). These results impel us to identify more such nutraceuticals which could promote brain health in neurodegenerative conditions. This chapter intends to describe the glial cell types involved in maintaining energy homeostasis as well as the role of glial cells and associated signaling pathways in mediating beneficial effects of naturally occurring immunomodulators (curcumin, blueberries, *Withania somnifera*, *Tinospora cordifolia*, *Bacopa monnieri*, *Ganoderma lucidum*, *Allium sativum*, and *Spirulina*) against neurodegenerative conditions. A more profound understanding of glia functioning will permit to identify their role both in healthy brain physiology and in pathological processes and may help in recognizing suitable therapeutic targets for the treatment of neurological disorders.

2 Glial Cells in Energy Homeostasis

The procedure of energy balance regulation is represented by the harmony between energy intake and expenditure, which gives a substrate to stable body weight support. The metabolic circuit development including hypothalamic neural projections is influenced by various nutritional and hormonal variables (Bouret et al. 2004, 2008). The physiological significance of such factor-based signaling pathway has also been elucidated in astrocytes and microglial cells (Crespo-Castrillo and Arevalo 2020).

Glial cells are considered as the *vanguard of neuroendocrinology* and contribute in physiological and pathophysiological mechanisms of systemic metabolism, appetite, and energy balance regulation. Specifically, astrocytes, microglia, and tanycytes have gained critical consideration in central control of body weight homeostasis and obesity (Yi et al. 2011; Pan et al. 2012; García-Cáceres et al. 2013, 2019). These nonneuronal cells are known for their ability to change their structure and function as per CNS microenvironment (Wolf et al. 2017; Verkhratsky and Nedergaard 2018). The severity of CNS damage governs the functional status of the glial cells, whereas glial activation in disease, injury, or damage aims to support neuronal survival and restore brain homeostasis by limiting neuronal damage. However, a prolonged and persistent activation of these cells adds to disease pathology (Robb et al. 2020). In other words, acute inflammation is important to prevent chronic inflammatory condition to maintain homeostasis. Various investigations have indicated changes in glial morphology and turnover following obesity (Gao et al. 2014; Schur et al. 2015), fasting (Zhang et al. 2017), hypoglycemia (McDougal et al. 2013), caloric restriction (Harrison et al. 2019), and protein malnutrition (Naik et al. 2017; Sinha et al. 2020b). These research findings extend generous confirmation that glial cells are directly or indirectly receptive to the dietary change (Robb et al. 2020).

2.1 The Warden Astrocytes

Astrocytes are associated with keeping up CNS homeostasis and advancing neuronal survival at all degrees of associations including molecular (ion, pH, water transport, and neurotransmitter homeostasis), cellular and network (neurogenesis, neuronal development and guidance, synaptogenesis, synaptic plasticity, maintenance and elimination), metabolic (neuro-glia vascular unit and glial-vascular interface, metabolic support, glycogen synthesis and storage), organ (blood-brain barrier and glymphatic system), and systemic homeostasis (chemosensing, energy balance, food intake, and sleep homeostasis; Verkhratsky and Nedergaard 2014, 2016, 2018). Pathological stimuli like tumors, trauma, aging, ischemia, stroke, and neurological disorders induce *astrocytic reactivity* with a shift in the phenotype of astrocytes. This is portrayed by alterations in gene expression accompanied with morphological and functional changes in astrocytes, which further lead to cellular hypertrophy, atrophy, proliferation, and scar formation. A few elements govern the astrocytic response including severity and nature of insult, level of blood-brain barrier (BBB) disruption, and inflammatory reaction (Matias et al. 2019).

Leptin hormone has emerged as a target for designing therapies to regulate metabolic functioning (Friedman and Mantzoros 2015). Different isoforms of leptin receptor are expressed by hypothalamic astrocytes, suggesting possible effects of leptin on glial cells (Hsuchou et al. 2009). Leptin is known to influence structural proteins and the potential of hypothalamic astrocytes to transport glutamate and glucose (Fuente-Martín et al. 2012), which further lead to alteration in excitability of neurons. Metreleptin (leptin mimetic) is used for the treatment of metabolic abnormalities like dyslipidemia, lipodystrophy, and ectopic accumulation of fat (Diker-Cohen et al. 2015; Vatier et al. 2016). The leptin receptors are effectively involved in functional cross talk with other signaling pathways to control energy balance. They also alter hypothalamic astrocyte morphology by changing number and length of astrocytic projections (García-Cáceres et al. 2011; Kim et al. 2014). Both leptin (anorexigenic hormone) and ghrelin (orexigenic hormone) are involved in modification of synaptic inputs to pro-opiomelanocortin (POMC) and neuropeptide Y (NPY) neurons in the arcuate nucleus (Pinto et al. 2004; Chowen et al. 2013).

Additionally, astrocytic leptin receptor knockout mice demonstrated remodeling of hypothalamic neuronal circuitry and attenuated leptin-induced anorexia (Jayaram et al. 2013; Kim et al. 2014; Rottkamp et al. 2015). Moreover, astroglial cells participate in the regulation of food intake via cognate receptors present on its surface like leptin receptor (LepR) and growth hormone secretagogue receptor (GHSR; Douglass et al. 2017). It is well acknowledged that feeding behavior in mammals is constrained by the neurons of medial basal hypothalamus. However, only few studies focused on glial relay circuit which controls feeding behavior. Yang et al. (2015) have demonstrated astrocytic bidirectional control of ghrelin- and leptin-mediated feeding behaviors by combining designer receptors exclusively activated by designer drugs (DREADD) approach with electrophysiology, pharmacology, and feeding assays. The mechanistic explanation for astrocytic inhibition of food consumption involves adenosine-mediated inactivation of orexigenic

agouti-related peptide (AGRP) neurons in hypothalamus arcuate nucleus (ARC). Astrocytic activation is reported to attenuate ghrelin-induced hyperphagia, though it advances leptin evoked anorexia and vice versa. In a way, astrocytes are now considered as emerging stars in maintaining whole-body energy homeostasis (Camandola 2018).

2.2 Tanycytes as Gatekeepers of CNS

Tanycytes are radial glia-like bipolar cells that line the floor and ventrolateral walls of the third ventricle (Goodman and Hajihosseini 2015). Tanycytes are further subtyped as α- and β-tanycytes according to their variations in localization, mechanism of action, and biological properties (Rodríguez et al. 2005). The privileged localization of tanycytes close to hypothalamic neurons makes it a suitable candidate to regulate energy balance, homeostasis, and appetite with diverse physiological functions. These cells are referred to as *gatekeepers* of CNS as they regulate BBB permeability together with capillary endothelia, release vascular endothelial growth factor A (VEGF-A), and selectively direct the access of molecules into CNS. They provide a critical channel that connects cerebrospinal fluid with neuroendocrine cells in arcuate and ventromedial nuclei (VMN) of hypothalamus (Argente-Arizon et al. 2016). These specialized ependymal cells act as nutritional conduits engaged in CNS glucoregulation by transporting glucose throughout BBB and third ventricle through glucose transporters 1 (GLUT1; Morgello et al. 1995). The α-subtype of tanycytes express GLUT2 and carries signals to glucose responsive neurons, i.e., glucose excited (GE) and glucose inhibited (GI) neurons in hypothalamic VMN and ARC. Apart from GLUT2 and glucokinase (GK), monocarboxylate transporters (MCT) inclusive of MCT1 and MCT4 are expressed by tanycytes, which participate in lactate influx and efflux (Cortés-Campos et al. 2011). Numerous studies have established the function of lactate in feeding behavior and glucosensing (Gerhart et al. 1998; Lam et al. 2005). The function of tanycytes in thyroid hormone metabolism is the most studied aspect of energy metabolism. The β-subunit of thyroid stimulating hormone (TSH) affects the deiodinase enzyme expression. This enzyme is responsible for converting thyroxine (T4) into triiodothyronine (T3) and accordingly plays an important role in the release of T3 and regulation of bioavailability of thyroid hormone in hypothalamus (Barrett et al. 2007; Ebling and Lewis 2018). These cells actively participate in transportation of specific metabolic signals throughout the BBB via extracellular signal regulated kinase (ERK) signaling pathway (Balland et al. 2014). These glial cells reorganize themselves and exert adaptive response as per the environmental need.

2.3 The Warrior Microglia

Microglial cells are exceptionally unique heterogeneously dispersed resident macrophages of the CNS and are known to function as *double edged sword*. They

have significant role in both physiological and pathophysiological conditions and exert either beneficial or detrimental effects. These cells are effectively engaged with programmed cell death, synaptic pruning, synapse maturation, and maintaining synaptic plasticity during development (Bilimoria and Stevens 2015), whereas in adult brains, they actively scan the brain parenchyma; phagocytose the invading pathogens; aid in clearing metabolic wastes, damaged neurons, and cellular debris; ensure neuronal survival; and maintain the CNS homeostasis (Mariani and Kielian 2009). Microglial activation, which is commonly accompanied by both increase in total cell count and morphological changes, is a major hallmark in majority of neurodegenerative disorders like Alzheimer's and Huntington's disease, multiple sclerosis, and amyotrophic lateral sclerosis (Cartier et al. 2014).

Microglial cells express transporters for all the available major energy substrates, i.e., glucose, glutamine, and fatty acids (Zhang et al. 2015). However, these cells use glucose as its significant fuel source and express different glucose transporters (GLUT1, GLUT3, GLUT4, GLUT5, GLUT6, GLUT8, GLUT9, GLUT10, GLUT12, and GLUT13) to ensure appropriate glucose influx under various circumstances. Among all available glucose transporters, GLUT1 is accounted to be expressed at the maximal level and control glycolytic pathway and phagocytosis, which are essential to maintain CNS integrity and homeostasis especially under inflammatory conditions (Wang et al. 2019). Glucose deprivation disturbs the microglial functioning by enhancing release of pro-inflammatory cytokines and promoting the phagocytic potential of microglia (Churchward et al. 2018).

In case of scarce or limited nutrient availability where carbohydrate and lipids are not adequate for proper energy supply, proteins can be utilized as metabolic substrate for energy production. Proteins are degraded to free amino acids via autophagic-lysosomal pathway (Kaur and Debnath 2015). The concentration-dependent impact of amino acids has been reported on microglial morphology and function, where physiological serine and glycine concentration in cerebrospinal fluid (CSF) promotes the appearance of ramified morphology of microglial cells with respect to standard culture media (Tanaka et al. 1998). Similarly, branched chain amino acids including valine, isoleucine, and leucine promote the appearance of intermediate phenotypic profile of microglial cells (De Simone et al. 2013). Microglial activation following infection or ischemic condition upregulates excitatory amino acid transporter-1 (EAAT-1) and glutamine synthetase (GS) expression to ensure neuronal survival and attenuate glutamate-induced excitotoxicity (Beschorner et al. 2007; Nakajima et al. 2016).

The glial cells are thus involved in maintaining metabolic homeostasis. Any disturbance in normal metabolic neuronal circuitry with respect to glia may lead to the progression of neurological disorders. It is evident that both *neurocentric* and *gliocentric vision* is necessary to increase our understanding on etiopathogenesis of neurological disorders. Along these lines, targeting glial cells for the treatment of neurological disorders offers promising way to design suitable pharmacological intervention against neurodegeneration.

3 Therapeutic Potential of Naturally Occurring Immunomodulators

A number of nutritional compounds have been identified with antioxidant and anti-inflammatory activities which could support both glial and neuronal survival. Both in vivo and in vitro studies have shown the effect of nutrients on microglial activation. In particular, resveratrol in grapes (Bi et al. 2005), epigallocatechin gallate in green tea (Li et al. 2004), and polyphenols in blueberries (Strömberg et al. 2005) inhibit microglial activation and protect against neuroinflammation. Glial cells respond to neuroinflammation by changing their structural, metabolic, and gene expression profile (Afridi et al. 2020). The majority of neurological and neurodegenerative conditions associated with aging and/or pathological insults arise from oxidative imbalance and neuroinflammation. These naturally occurring immunomodulators may exert beneficial effects against astrocytosis, microgliosis, apoptosis, oxidative stress, and neuroinflammation and, thus, could be used as a promising approach against neurodegenerative disorders (summarized in Fig. 23.1).

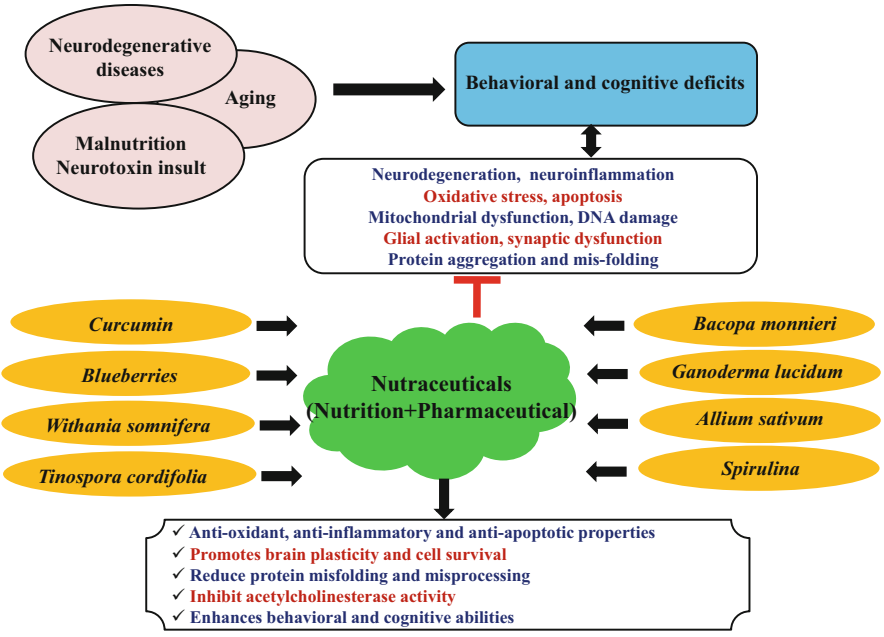

Fig. 23.1 Overview of possible targets for nutraceuticals in promoting neurocognition: Nutraceuticals may exert beneficial effects against neurodegenerative conditions by inhibiting glial activation, oxidative stress, and neuroinflammation and ameliorating associated behavioral and cognitive deficits

3.1 Curcumin

Curcumin is a major phenolic compound isolated from rhizome of the turmeric (*Curcuma longa*). It is well known for its anti-inflammatory, antioxidative, antitumor, anti-amyloidogenic, hepatoprotective, analgesic, antidiabetic, antiviral, antibacterial, antimutagenic, vasculoprotective, cardioprotective, pulmonoprotective, and immunomodulatory properties (Ghasemi et al. 2019). Apart from its medicinal value, it is used in the food and cosmetic industries also. Accumulating evidences suggest neuroprotective effects of curcumin on both neuronal and glial cells especially in the context of neuroinflammation (Cole et al. 2007; Hosseini and Hosseinzadeh 2018; Mukherjee et al. 2018). Curcumin exerts such beneficial effect via mitogen activated protein kinase (MAPK), NFκβ, WNT/β-catenin, P13k/Akt, STAT 3 signaling (Momtazi et al. 2016; Hesari et al. 2018) and activating heme-oxygenase 1 (HO-1), nuclear factor erythroid 2-related factor 2 (Nrf-2), and antioxidant response element (ARE) mechanisms (Cianciulli et al. 2016; Ding et al. 2016; Abdollahi et al. 2018). It inhibits microglial activation and blocks expression of inducible nitric oxide synthase (iNOS), tumor necrosis factor alpha (TNFα), reactive nitrogen species (RNS), cyclooxygenase-2 (COX-2), and lipoxygenase (LOX) (Parada et al. 2015; Eun et al. 2017; Akaishi and Abe 2018).

Curcumin downregulates the expression of glial fibrillary acidic protein (GFAP) and prevents the activation and mitochondrial dysfunction in astrocytes (Daverey and Agrawal 2016). It has been reported in a recent study that curcumin administration at a dose of 50 mg/kg body weight prevents LPS-induced neuroinflammation and long-term memory impairment (Sorrenti et al. 2018). The therapeutic benefits of curcumin have also been reported in animal models of Parkinson's disease, (PD) Alzheimer's disease (AD), Huntington's disease (HD), multiple sclerosis, depression, and schizophrenia (Lee et al. 2013). Curcumin supplementation at a dose of 100 mg/kg body weight to pentylenetetrazole (PTZ) treated Wistar rats reduces mRNA and protein expression of both GFAP and Iba-1 in the hippocampus and cortex and maintains the glial population in resting state, evidencing therapeutic potential of curcumin to suppress glial activation and neuroinflammation in chronic epilepsy (Kaur et al. 2015). Some studies also reported that curcumin effectively inhibits lipoteichoic acid (LTA) induced production of neuroinflammatory molecules (COX-2 and iNOS) and activation of NFκβ in BV2 microglial cells, further suggesting the therapeutic efficacy of curcumin against neuroinflammation associated disorders (Yu et al. 2018).

3.2 Blueberries

Blueberries are potent source of flavonoids including caffeic acids, flavanols, anthocyanins, and hydroxycinnamates (Gavrilova et al. 2011; You et al. 2011). The biological functions of blueberries majorly include antioxidant, anti-inflammatory, and cardiovascular properties. Such beneficial effects of blueberry

supplementation are attributed via MAPK, extracellular signal regulated kinase (ERK), and cyclic-AMP response element binding protein (CREB) mechanisms (Subash et al. 2014). These mechanisms are also actively involved in upregulating learning and memory at synaptic sites. There is evidence that blueberry consumption by F344 male rats effectively dropped OX-6 positive microglia in the striatum and globus pallidus (Strömberg et al. 2005). Willis and associates in 2010 reported reduced astrogliosis and microglial activation along with reduction in OX-6 microglial immunoreactivity and pro-inflammatory IL-6 level in intraocular hippocampal grafts following 2% blueberry diet. IL-6 has been found to affect neuronal division and maturation (Erta et al. 2012).

Dietary supplementation with blueberry extract exerts rescuing effect against cellular damage and oxidative stress induced by hippocampal injections of kainic acid (Duffy et al. 2008) and prevents amyloid beta accumulation and behavioral deficits in Alzheimer's disease animal model (Joseph et al. 2003). Moreover, the blueberry supplementation has been reported to positively affect the survival of dopaminergic neurons in PD model (McGuire et al. 2006). The neuroprotective effect of blueberry supplementation was also reported by Çoban and associates in 2015 in a study showing that blueberry supplementation attenuates apoptosis, reduces malondialdehyde and acetylcholinesterase activity, and increases antioxidant enzyme activity with histopathological amelioration in rat brain treated with D-galactose.

Furthermore, Sweeney et al. (2002) found that 6 weeks of dietary supplementation of blueberry reduces hippocampal neuronal loss in rats with cerebral ischemia. Interestingly, dietary intake of blueberry also reverses the age associated cognitive and behavioral deficits (Joseph et al. 1999). In other study, Papandreou et al. (2009) found that supplementation of polyphenol-rich wild blueberry extract to healthy mice enhances brain ascorbate and glutathione levels and inhibits acetylcholinesterase activity. It has been demonstrated that blueberry extract treatment reduces ischemia/reperfusion induced apoptosis and cerebral infarction (Wang et al. 2005). According to Debnath-Canning and associates (2020), cell culture of microglia when exposed to glutamate or α-synuclein results in microglial activation. Interestingly, blueberry fruit and leaf extract when added to cells treated with glutamate or α-synuclein causes increase in overall number of viable cells along with reduction in population of activated microglial cells. These beneficial neuroprotective effects of blueberry could be attributed to the presence of polyphenolic compounds such as anthocyanins, which demonstrate high antioxidant defense capacity, thereby making it suitable candidate for neuroprotection against neurodegenerative disorders.

These research studies indicate that blueberry supplementation exerts critical beneficial impact on brain function by decreasing pro-inflammatory cytokine release, oxidative stress, microglial activation, astrogliosis, and shift in the morphology of glial cells toward resting state and, thus, promotes neuronal survival and behavioral functions.

3.3 *Withania somnifera*

Withania somnifera (WS) (commonly called *Ashwagandha*, *Indian ginseng*, *winter cherry*) is a traditional Indian herb of family Solanaceae and belongs to *medhyarasayanas* (medhya refers to mind) and, thus, is considered as nootropic herb and/or nervine tonic. Ashwagandha is one of the most valuable herbs of Indian Ayurvedic system of medicine and is well known for its health promoting effects and enhancement of cognitive functions (Singh et al. 2011). Phyto-pharmacological studies showed that WS extract possesses immunomodulatory (Archana and Namasivayam 1998; Trivedi et al. 2017), anti-inflammatory (Bhattacharya et al. 1997), anticancer (Rai et al. 2016), antioxidant (Dhuley 1998), anti-stress (Ziauddin et al. 1996), rejuvenating, and life prolonging properties (Akhoon et al. 2016) and cognition promoting effect (Singh and Udupa 1993) and also improved cell mediated immunity (Singh et al. 2011). The neuroprotective and neuroregenerative potential of WS has been reported in animal models of neurodegeneration including PD, AD, HD, and cerebral ischemia (Bhatnagar et al. 2009; Kuboyama et al. 2014).

The important biological active constituents of WS include alkaloids (withanine, somniferine, somnine, somniferinine, withananine, psuedo-withanine, isopelletierine, tropine, psuedotropine, 3-α-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine, and anahydrine), steroidal lactones (steroidal lactones, withaferin A, withanolides A–Y, withasomniferin-A, withasomidienone, withasomniferols A–C, withanone), saponins, and flavonoids (Atta-Ur-Rahman et al. 1991; Choudhary et al. 1995; Kulkarni and Dhir 2008; John 2014; Dar et al. 2017). Alkaloids and lactones are collectively known as withanolides (Elsakka et al. 1990). Numerous studies indicate that Ashwagandha prevents synaptic loss and neuritic atrophy and promotes neuritic outgrowth, which is attributed to the presence of active ingredients in WS, such as, withanolide A, withanoside IV, and withanoside VI (Zhao et al. 2002). Ashwagandha root extract also possesses GABA mimetic activity, which could be beneficial in GABAergic neurodegeneration and tardive dyskinesia (Gunne and Andrén 1993). Kuboyama et al. (2006) showed that oral administration of withanoside IV in mice prevents axonal, dendritic, and synaptic loss and promotes synaptogenesis, axonal and dendritic outgrowth, and cognitive functions in Aβ (25-35)-induced neurodegeneration. WS also shows beneficial effects in clinical conditions of depression and anxiety and exerts anxiolytic and antidepressant effect (Abdel-Magied et al. 2001).

Neuroprotective effects of alcoholic extract of Ashwagandha were also reported against scopolamine induced amnesia and associated alterations in molecules governing neuronal and glial plasticity, by upregulating the expression of brain-derived neurotrophic factor (BDNF), GFAP, neurofilament-H (NF-H), microtubule associated protein (MAP2), postsynaptic marker protein (PSD-95), and growth associated protein-43 (GAP-43), decreasing the activity of H2A family member X (γH2AX) and reactive oxygen species (ROS). Such modulation assists in recovery of synaptic functions and protects against scopolamine induced DNA damage and oxidative stress (Konar et al. 2011). Shah et al. (2009) performed an intriguing study to assess the differentiation potential of alcoholic extract of Ashwagandha (i-extract)

and its different constituents including withaferin A, withanone, and withanolide A in rat and human glioma cell lines and observed that i-extract and its constituents effectively inhibit the proliferation of glioma cells. Shah and associates (2009) further observed other dose-dependent changes like apoptosis, growth arrest, morphological switch of astrocytes to activated states, and induction of senescence along with decrease in motility and adhesion, thereby suggesting efficacy of WS extract and its components in glioma treatment. Another study by the same group of researchers (Shah et al. 2015) reported the therapeutic efficacy of water and alcoholic leaf extract of Ashwagandha against glutamate-induced cytotoxicity and oxidative damage caused by hydrogen peroxide, where increased GFAP, NF-200, and MAP-2 proteins might be attributed in promoting the survival of neuronal and glial cells.

Ashwagandha leaf water extract (ASH-WEX) also prevents LPS-induced microglial activation and nitro-oxidative stress and inhibits the release of pro-inflammatory cytokines (Gupta and Kaur 2016). The contributing factors for anti-inflammatory activity of Ashwagandha were found to be NFkβ, P38, JNK, and MAPK pathways (Gupta and Kaur 2018). ASH-WEX supplementation to LPS treated rats was also reported to ameliorate neuromuscular coordination and cognitive functions and prevent neuronal apoptosis, thus indicating neuroprotective role of ASH-WEX against inflammation mediated neurodegeneration (Gupta and Kaur 2019). From these research studies, it is clear that Ashwagandha is the *Queen of Ayurveda* and possesses enormous therapeutic potential as nutraceutical and/or pharmaceutical against neuron and glia degeneration.

3.4 *Tinospora cordifolia*

Tinospora cordifolia (commonly called as *Guduchi* in Ayurveda) is a deciduous climbing shrub of family Menispermaceae and belongs to *medhyarasayanas*, thus used as a cognitive enhancer. In Hindi, it is called as *Giloya*, which is a Hindu mythological term for *heavenly elixir* or *Amrita*. It is well known for its immense medicinal properties including antidiabetic, anti-stress, antioxidant, anti-inflammatory, anti-arthritic, hepatoprotective, radioprotective, neuroprotective, and immunomodulatory activities and also promotes longevity and improved body health (Saha and Ghosh 2012; Pandey et al. 2020). Its therapeutic properties are due to the presence of biologically active ingredients like diterpenoid lactones, glycosides, steroids, sesquiterpenoid, flavonoids, phenolics, aliphatic compounds, essential oils, a mixture of fatty acids, and polysaccharides, berberine, and palmatine (Shirolkar et al. 2016; Sharma et al. 2019). The glycosides (furanoid diterpene glucoside, tinocordiside, tinocordifolioside, cordioside) isolated from stem of *T. cordifolia* have been reported to treat ALS (amyotrophic lateral sclerosis), attention deficit hyperactivity disorder (ADHD), PD, dementia, and motor and cognitive deficits (Jahfar 2003; Mutalik and Mutalik 2011; Saha and Ghosh 2012; Sharma et al. 2020). In addition, it has been reported that extracts of *T. cordifolia* prevents oxidative brain damage during oxygen-glucose deprivation (Rawal et al. 2004). Report on phytotherapeutic potential of *T. cordifolia* against glioblastoma also exists

where ethanolic extract of *T. cordifolia* (TCE) showed proapoptotic, antiproliferative, and differentiation and senescence inducing potential in C6 glioma cells (Mishra and Kaur 2013).

It is reported that Guduchi is also effective against alcoholism. Chronic alcohol consumption is associated with hyperlipidemia, oxidative stress, and disturbed dopamine metabolism. Guduchi treatment significantly ameliorates alcoholism induced impaired lipid metabolism and oxidative burden and activates PPARα (proliferator activated receptor), CREB, and SREBP-1 (sterol regulatory element binding protein) by targeting dopaminergic neurotransmission. DDR1 (dopamine D1 receptor) agonist in Guduchi activates cAMP synthesis resulting in protein kinase A activation, which further assists in phosphorylation of PPARα, thus indicating anti-alcoholism and hypolipidemic activity of Guduchi (Shirolkar et al. 2016). More recently, Birla et al. (2019) reported anti-inflammatory activity of *T. cordifolia* in 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine (MPTP) intoxicated Parkinsonian mouse model. Similarly, oral administration of TCE in 6-hydroxy dopamine (6-OHDA) induced Parkinson model of rat enhances the dopamine level, reduces oxidative stress, and improves exploratory activity (Kosaraju et al. 2014). Recently, the neuroprotective activity of TCE against LPS-induced neuroinflammation was reported by Prakash et al. (2017), where TCE treated Wistar rats showed poor pro-inflammatory cytokine levels (TNFα, IL-1β, IL-6) and positively modulated antioxidant defense enzyme activity (SOD, CAT, GSH), reducing neuronal damage. In a similar way, butanol extract of *T. cordifolia* was found to exert neuroregenerative effects against glutamate-induced excitotoxicity by positively modulating the pathways governing neuronal differentiation, apoptosis, and homeostasis (Sharma and Kaur 2018).

Antidepressant activity of *T. cordifolia* was also observed in Swiss mice, where administration of petroleum ether extracts of *T. cordifolia* for 14 days to mice increased brain monoamines and reverses the depressive-like behavior in tail suspension test and forced swim test (Dhingra and Goyal 2008).

Likewise, memory enhancer and neuro-immunomodulatory potential of TCE was studied in TCE treated sleep deprived rats and noted the TCE was able to suppress anxiety-like behavior and restore exploratory behavior and cognitive functions. The mechanistic explanation for observed neurobehavioral function could be poor expression of inflammatory markers (CD11b/c, MHC-I, cytokines), positively modulated activity of proteins responsible for synaptic plasticity (NCAM, GAP-43) and LTP maintenance (CaMKII-α, calcineurin), reduced oxidative damage and apoptosis, and suppressed activation of glial cells (Mishra et al. 2016). Thus, consumption of *T. cordifolia* extract may provide effective therapeutics for the treatment of neurodegenerative disorders and mild cognitive impairment by regulating activity of glial cells and cytokine release and preventing oxidative burden.

3.5 *Bacopa monnieri*

Bacopa monnieri (BM; commonly called as Brahmi, Bramabhi, Nirabarhmi, water hyssop) is a perennial herbaceous plant of family Scrophulariaceae and is considered as one of the most important medicinal plants in Indian traditional medicine system. It belongs to the class *medhyarasayanas* and is widely used as a neurotonic and memory enhancer. Various active constituents are present in Brahmi including saponins, bacosides A and B, flavonoids, alkaloids, brahmine, betulinic acid, nicotine, α-alanine, aspartic acid, glutamic acid, serine, pseudojujubogenin glycoside, and herpestine (Deepak and Amit 2004; Shinomol 2011; Devishree et al. 2017), which makes it a suitable candidate for modulating the brain functions and behavior. *Bacopa* has been extensively used for maintaining overall body health and possesses antidepressant, antioxidant, antianxiety, antiepileptic, adaptogenic, sedative, analgesic cardiovascular, antidiabetic, anti-arthritic, anticancer, antihypertensive, antilipidemia, anti-inflammatory, hepatoprotective, and neuroprotective properties (Jeyasri et al. 2020). Aguiar and Borowski 2013 reported the active involvement of *Bacopa* in inhibiting acetylcholinesterase activity (AChE) and β-amyloid accumulation and modulating neurotransmitter activity and cerebral blood flow. The nootropic functions of Brahmi are extensively reviewed by Stough et al. (2013) and Kongkeaw et al. (2014). The alkaloid extract of *Bacopa* targets the inflammatory pathways in the brain and effectively inhibits the release of inflammatory cytokines, TNFα, and IL-6 from LPS-activated microglial cells and downregulates the enzymes (caspase-1 and caspase-3, matrix metalloproteinase-3) associated with inflammation (Nemetchek et al. 2017). In a similar way, BM extracts (CDRI-08) upregulate the expression of neuronal (BDNF, Arc) and glial (GFAP) plasticity markers in scopolamine induced amnesic mice, thus indicating the anti-amnesic potency of Brahmi (Konar et al. 2015).

The neuroprotective potential of BM against hydrogen peroxide induced oxidative damage was also reported by Bhatia et al. (2017), where the methanolic extract of BM (BM-MEx) was found to alleviate the expression of astrocytic cytoskeletal protein (GFAP) and stress markers (HSP70 and Grp75) and modulates the antioxidant defense enzyme activity in C6 glioma cell culture. The additive effects of BM with other herbal extracts were also observed where the combination of BM and rosemary exerts more beneficial effects than either agent alone. The combined extract enhances BDNF levels and antioxidant activity and inhibits the tau phosphorylation on human glial and mouse hypothalamus cells (Ramachandran et al. 2014). Its consumption also inhibits the degeneration of cholinergic neurons and prevents amyloid formation, suggesting cholinergic and anti-amyloidogenic potential of BM, respectively, and, thus, it could be used as a therapeutic agent against Alzheimer's disease (Mathew and Subramanian 2012). To elucidate the cellular and molecular mechanisms underlying the nootropic potency of BM, RNA seq approach was used to identify transcriptomic alterations on SH-SY5Y human neuroblastoma cells. The analysis revealed that BM positively regulates mRNA translation, membrane transport, and responses to oxidative damage and protein misfolding, which are the key determinants of its nootropic effect (Leung et al. 2017). Majority of the

brain disorders are associated with the disturbed neuroinflammatory components; thus, targeting the neuroinflammatory pathways using herbal formulations may open up new avenues to treat neurodegenerative diseases like AD, PD, multiple sclerosis, and psychiatric disorders like anxiety, depression, and schizophrenia.

3.6 *Ganoderma lucidum*

Ganoderma lucidum (GL; also known as Lingzhi or Reishi) is a medicinal mushroom which belongs to Polyporaceae family of Basidiomycota. It has been used in traditional Chinese and Japanese medicine as a herbal remedy for thousands of years. Its health promoting effects are associated with the presence of bioactive compounds, such as polysaccharides, proteins, and triterpenoids in fruiting bodies, spores, and cultured mycelia (Mizushina et al. 1998). It is also considered as *the mushroom of immortality* or *the king of herbs* because of the numerous beneficial health effects associated with its consumption (Khatian and Aslam 2019). Diverse pharmacological actions of GL have been reported which prominently include antioxidant, anti-inflammatory, antitumor, antimicrobial, hepatoprotective, anti-arthritic, antiaging, and immunomodulation properties (Ahmad 2018). Numerous studies have evidenced the protective role of GL in AD, PD, HD, and epilepsy (Chen et al. 2008; Lai et al. 2008). The extracts of GL (GLE) prevent the degeneration of dopaminergic neurons by inhibiting the microglial activation and suppressing the release of microglia derived pro-inflammatory cytokines (TNFα, IL-1β). This efficacy of GL in preventing neurodegeneration in PD was reported by Zhang et al. (2011). The polysaccharide of *G. lucidum* (GLP) exhibits neuroprotective effects against spinal cord injury by decreasing caspase-3 activity, TNFα, MDA, and nitric oxide levels (Gokce et al. 2015).

Other studies also revealed that GLP enhances the cell viability of cortical neurons following hypoxia/reoxygenation injury in rats (Zhao et al. 2004). Moreover, GL targets the different signaling pathways like ras/ERK and CREB to regulate the survival of neuronal and glial cells (Cheung et al. 2000). Its extract also reactivates AMPK/Mtor/ULK1 and PINK1/Parkin pathways which are actively involved in regulating mitochondrial functioning as well as autophagic response and thus assists in ameliorating Parkinsonism pathology (Ren et al. 2019). Thus, high nutritive and medicinal value of GL makes it a suitable agent against neurodegenerative conditions. However, only few studies showed the possible impact of GLE on glial cells. Thus, future studies are needed to determine the glioprotective potential of *G. lucidum*.

3.7 *Allium sativum*

Allium sativum, commonly called as garlic, has been widely used for medicinal purposes since ancient times. It has been observed that aged garlic extract (AGE) prevents oxidative imbalance by scavenging reactive oxygen species and

suppressing the lipid peroxide formation (Morihara et al. 2011). AGE is also known to produce various stable organosulfur compounds including S-allyl cysteine (SAC) and thiosulfinates, which exert numerous health benefits through antioxidant, anti-inflammatory, and antiapoptotic activities (Chauhan 2006). Despite well-known role of garlic in neuroprotection, only a handful of studies focused on the impact of garlic treatment on glial cells. Nillert and associates in 2017 demonstrated neuroprotective effects of AGE treatment at doses of 125, 250, and 500 mg/kg body weight on Aβ (1-42)-induced neuroinflammation and cognitive dysfunction. AGE exerts beneficial effect by reducing the microglial activation, CD11b immunoreactivity, and IL-1β levels in the hippocampus region of rat brain and thereby assists in amelioration of the short-term recognition memory. In addition to antioxidant and anti-inflammatory effect, garlic extract targets multiple pathways in order to improve neurodegenerative conditions. The therapeutic efficacy has been explored in various conditions of neurodegeneration (Chauhan 2006; Mukherjee and Banerjee 2013).

Another poorly explored component in AGE is N-α-(1-deoxy-D-Fructos-1-yl)-L arginine (FruArg). It regulates neuroinflammation by targeting the number of signaling pathways like Toll-like receptor, IL-6 signaling, and Nrf-2. Proteomic analysis revealed that FruArg regulates neuroinflammatory response and promotes resilience in LPS-activated murine BV-2 microglial cells by targeting proteins associated with oxidative stress and suppressing nitric oxide production (Zhou et al. 2014). Additionally, AGE downregulates the expression levels of various neuroinflammatory mediators like IL-1β, TLR-4, Nrf-2, and heme-oxygenase (HO-1); positively modulates antioxidant signaling enzymatic activity including SOD, glutathione peroxidase (GPX), and glutathione (GSH); and also reduces the activation of astrocytes and microglial cells (Ide and Lau 1999; Hiramatsu et al. 2016; Song et al. 2016; Thomson et al. 2015). Importantly, garlic extract is a highly promising therapeutic candidate for treating oxidative stress and neuroinflammation related neurodegenerative disorders

3.8 Spirulina

Spirulina microalgae is well known for its antioxidant, anti-inflammatory, immunomodulatory, and neuroprotective properties. It is widely used as a nutritional supplement as it contains proteins, vitamins, minerals, essential fatty acids, polysaccharides, and various pigments like phycocyanin and carotenoids (Sinha et al. 2018). Considerably strong evidences are available, showing decreased glial activation following *Spirulina* consumption (Sinha et al. 2020a, b). It has been observed that consumption of 0.1% *Spirulina* supplemented diet for 28 days before LPS administration prevents astrogliosis (Bachstetter et al. 2010). Patro and associates in 2011 reported that *Spirulina platensis* treatment (400 mg/kg) to collagen induced arthritic rats potentially inhibits microglial activation in dorsal and ventral horns of spinal cord, ameliorates motor coordination and sciatic functional index, and restores functional motor recovery.

Previous studies also demonstrated that blueberry and *Spirulina* enriched diets reduced MHC-11 expressing cells and also improved striatal dopamine recovery (Strömberg et al. 2005). In a similar study by Pabon and associates (2012), neuroprotective effects of *Spirulina* enhanced diet in an α-synuclein model of PD was also reported, where *Spirulina* effectively decreased the expression of MHC-11 expressing cells (marker linked with M1 phenotype of microglial activation).

Our lab also contributed in understanding the impact of gestational protein malnutrition and *Spirulina* supplementation on neuroinflammatory response in F1 progeny of rats. *Spirulina* supplementation (400 mg/kg/b.wt.) to protein malnourished pregnant and lactating dams partially prevents protein malnutrition induced oxidative stress, reactive gliosis, and neurodegeneration. With respect to glia, *Spirulina* supplementation suppressed the malnutrition-associated astrogliosis by downregulating the expression of GFAP and S100β proteins. Moreover, oral administration of *Spirulina* also partially attenuated the malnutrition induced increase in OX-6, OX-42, and Iba-1 immunostaining, thereby representing the neuroprotective potential of *Spirulina* in positively modulating the protein deprivation induced reactive astrogliosis and microgliosis (Sinha et al. 2020b).

Neurodegeneration is generally marked by the failure of important functions of glia, i.e., maintenance of overall homeostasis in CNS, neuronal metabolism, synaptic plasticity, and associated reactive gliosis (Verkhratsky et al. 2014). In particular, astrocytic and microglial activation is linked with pathophysiology of neurodegenerative diseases. Natural products like *Spirulina* may reverse these effects due to its antioxidant/anti-inflammatory properties.

4 Concluding Remarks

This chapter highlights the role of neuro-nutraceuticals in regulating neuron-glia cross talk and the need to target therapeutic strategies that are beneficial to behavioral and cognitive health of an individual. The currently available strategies for the treatment of neurodegenerative conditions are not sufficient to fully cure the disease. Certainly, these nutraceuticals can work synergistically with other herbal agents and can be used for designing polyherbal formulations for better management of neurological conditions. Unfortunately, a very few studies elucidated the effects of herbal compounds on glial cells. The exact mechanism by which nutraceuticals inhibit glial activation is not known. However, their antioxidant and anti-inflammatory activities seem to play glioprotective and neuroprotective role. Thus, it would be right to conclude that natural immunomodulators may be effective in preventing and treating neurodegenerative conditions. Future studies will critically address how dietary intake of neuro-nutraceuticals would influence glial activation and up to what degree their consumption would alleviate the severity and symptoms of neurodegenerative diseases.

Acknowledgment Department of Biotechnology's support through the DBT-National Initiative on Glial Cell Research in Health and Disease is gratefully acknowledged. We are thankful to the

Department of Biotechnology, Department of Science and Technology, and the Indian Council of Medical Research, Govt. of India, for their financial support through various grants. We are thankful to Dr. Urmishree Bedamata for critically evaluating the manuscript for its English.

References

Abdel-Magied EM, Abdel-Rahman HA, Harraz FM (2001) The effect of aqueous extracts of Cynomorium coccineum and Withania somnifera on testicular development in immature Wistar rats. J Ethnopharmacol 75(1):1–4

Abdollahi E, Momtazi AA, Johnston TP, Sahebkar A (2018) Therapeutic effects of curcumin in inflammatory and immune-mediated diseases: a nature-made jack-of-all-trades? J Cell Physiol 233(2):830–848

Afridi R, Kim JH, Rahman MH, Suk K (2020) Metabolic regulation of glial phenotypes: implications in neuron–glia interactions and neurological disorders. Front Cell Neurosci 14:20

Aguiar S, Borowski T (2013) Neuropharmacological review of the nootropic herb Bacopa monnieri. Rejuvenation Res 16(4):313–326

Ahmad MF (2018) Ganoderma lucidum: Persuasive biologically active constituents and their health endorsement. Biomed Pharmacother 107:507–519

Akaishi T, Abe K (2018) CNB-001, a synthetic pyrazole derivative of curcumin, suppresses lipopolysaccharide-induced nitric oxide production through the inhibition of NF-κB and p38 MAPK pathways in microglia. Eur J Pharmacol 819:190–197

Akhoon BA, Pandey S, Tiwari S, Pandey R (2016) Withanolide A offers neuroprotection, ameliorates stress resistance and prolongs the life expectancy of Caenorhabditis elegans. Exp Gerontol 78:47–56

Archana R, Namasivayam A (1998) Antistressor effect of Withania somnifera. J Ethnopharmacol 64(1):91–93

Argente-Arizon P, Guerra-Cantera S, Garcia-Segura LM, Argente J, Chowen JA (2016) Glial cells and energy balance. J Mol Endocrinol 58(1):R59–R71

Atta-Ur-Rahman S, Arshad Jamal M, Choudhary I, Asif E (1991) Two withanolides from Withania somn. Phytochemistry 30:3824–3826

Bachstetter AD, Jernberg J, Schlunk A, Vila JL, Hudson C, Cole MJ, Shytle RD, Tan J, Sanberg PR, Sanberg CD, Borlongan C (2010) Spirulina promotes stem cell genesis and protects against LPS induced declines in neural stem cell proliferation. PLoS One 5(5):e10496

Balland E, Dam J, Langlet F, Caron E, Steculorum S, Messina A, Rasika S, Falluel-Morel A, Anouar Y, Dehouck B, Trinquet E (2014) Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. Cell Metab 19(2):293–301

Barrett P, Ebling FJ, Schuhler S, Wilson D, Ross AW, Warner A, Jethwa P, Boelen A, Visser TJ, Ozanne DM, Archer ZA (2007) Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. Endocrinol 148(8):3608–3617

Beschorner R, Dietz K, Schauer N, Mittelbronn M, Schluesener HJ, Trautmann K, Meyermann R, Simon P (2007) Expression of EAAT1 reflects a possible neuroprotective function of reactive astrocytes and activated microglia following human traumatic brain injury. Histol Histopathol 22:515–526

Bhatia G, Dhuna V, Dhuna K, Kaur M, Singh J (2017) Bacopa monnieri extracts prevent hydrogen peroxide-induced oxidative damage in a cellular model of neuroblastoma IMR32 cells. Chin J Nat Med 15(11):834–846

Bhatnagar M, Sharma D, Salvi M (2009) Neuroprotective effects of Withania somnifera dunal.: a possible mechanism. Neurochem Res 34(11):1975–1983

Bhattacharya SK, Satyan KS, Ghosal S (1997) Antioxidant activity of glycowithanolides from Withania somnifera. Indian J Exp Biol 35:236–239

Bi XL, Yang JY, Dong YX, Wang JM, Cui YH, Ikeshima T, Zhao YQ, Wu CF (2005) Resveratrol inhibits nitric oxide and TNF-α production by lipopolysaccharide-activated microglia. Int Immunopharmacol 5(1):185–193
Bilimoria PM, Stevens B (2015) Microglia function during brain development: new insights from animal models. Brain Res 1617:7–17
Birla H, Rai SN, Singh SS, Zahra W, Rawat A, Tiwari N, Singh RK, Pathak A, Singh SP (2019) Tinospora cordifolia suppresses neuroinflammation in parkinsonian mouse model. Neuromolecular Med 21(1):42–53
Bouret SG, Draper SJ, Simerly RB (2004) Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 304(5667):108–110
Bouret SG, Gorski JN, Patterson CM, Chen S, Levin BE, Simerly RB (2008) Hypothalamic neural projections are permanently disrupted in diet-induced obese rats. Cell Metab 7(2):179–185
Camandola S (2018) Astrocytes, emerging stars of energy homeostasis. Cell stress 2(10):246
Cartier N, Lewis CA, Zhang R, Rossi FM (2014) The role of microglia in human disease: therapeutic tool or target? Acta Neuropathologica 128(3):363–380
Chauhan NB (2006) Effect of aged garlic extract on APP processing and tau phosphorylation in Alzheimer's transgenic model Tg2576. J Ethnopharmacol 108(3):385–394
Chen Y, Zhu SB, Xie MY, Nie SP, Liu W, Li C, Gong XF, Wang YX (2008) Quality control and original discrimination of Ganoderma lucidum based on high-performance liquid chromatographic fingerprints and combined chemometrics methods. Analytica Chimica Acta 623(2):146–156
Cheung WM, Hui WS, Chu PW, Chiu SW, Ip NY (2000) Ganoderma extract activates MAP kinases and induces the neuronal differentiation of rat pheochromocytoma PC12 cells. FEBS Lett 486(3):291–296
Choudhary MI, Parveen Z, Jabbar A, Ali I (1995) Antifungal steroidal lactones from Withania coagulance. Phytochemistry 40(4):1243–1246
Chowen JA, Argente J, Horvath TL (2013) Uncovering novel roles of nonneuronal cells in body weight homeostasis and obesity. Endocrinology 154(9):3001–3007
Churchward MA, Tchir DR, Todd KG (2018) Microglial function during glucose deprivation: inflammatory and neuropsychiatric implications. Mol Neurobiol 55(2):1477–1487
Cianciulli A, Calvello R, Porro C, Trotta T, Salvatore R, Panaro MA (2016) PI3k/Akt signalling pathway plays a crucial role in the anti-inflammatory effects of curcumin in LPS-activated microglia. Int Immunopharmacol 36:282–290
Çoban J, Dogan-Ekici I, Aydın AF, Betul-Kalaz E, Dogru-Abbasoglu S, Uysal M (2015) Blueberry treatment decreased D-galactose-induced oxidative stress and brain damage in rats. Metab Brain Dis 30(3):793–802
Cole GM, Teter B, Frautschy SA (2007) Neuroprotective effects of curcumin. The molecular targets and therapeutic uses of curcumin in health and disease:197–212
Cortés-Campos C, Elizondo R, Llanos P, Uranga RM, Nualart F, García MA (2011) MCT expression and lactate influx/efflux in tanycytes involved in glia-neuron metabolic interaction. PloS one 6(1):e16411
Crespo-Castrillo A, Arevalo MA (2020) Microglial and astrocytic function in physiological and pathological conditions: estrogenic modulation. Int J Mol Sci 21(9):3219
Dadhania PV, Trivedi PP, Vikram A, Tripathi ND (2016) Nutraceuticals against neurodegeneration: a mechanistic insight. Curr Neuropharmacol 14(6):627–640
Dantuma E, Merchant S, Sugaya K (2010) Stem cells for the treatment of neurodegenerative diseases. Stem Cell Res Ther 1(5):1–7
Dar NJ, Bhat JA, Satti NK, Sharma PR, Hamid A, Ahmad M (2017) Withanone, an active constituent from Withania somnifera, affords protection against NMDA-induced excitotoxicity in neuron-like cells. Mol Neurobiol 54(7):5061–5073
Daverey A, Agrawal SK (2016) Curcumin alleviates oxidative stress and mitochondrial dysfunction in astrocytes. Neuroscience 1333:92–103

De Simone R, Vissicchio F, Mingarelli C, De Nuccio C, Visentin S, Ajmone-Cat MA, Minghetti L (2013) Branched-chain amino acids influence the immune properties of microglial cells and their responsiveness to pro-inflammatory signals. Biochimica et Biophysica Acta (BBA)-Mol Basis Dis 1832(5):650–659

Debnath-Canning M, Unruh S, Vyas P, Daneshtalab N, Igamberdiev AU, Weber JT (2020) Fruits and leaves from wild blueberry plants contain diverse polyphenols and decrease neuroinflammatory responses in microglia. J Funct Foods 68:103906

Deepak M, Amit A (2004) The need for establishing identities of bacoside A and B', the putative major bioactive saponins of Indian medicinal plant Bacopa monnieri. Phytomed 11(2/3):264

Devishree RA, Kumar S, Jain AR (2017) Short term effect of Bacopa monnieri on memory—a brief review. J Pharm Res 11:1447–1450

Dhingra D, Goyal PK (2008) Evidences for the involvement of monoaminergic and GABAergic systems in antidepressant-like activity of Tinospora cordifolia in mice. Indian J Pharm Sci 70(6): 761

Dhuley JN (1998) Effect of ashwagandha on lipid peroxidation in stress-induced animals. J Ethnopharmacol 60(2):173–178

Diker-Cohen T, Cochran E, Gorden P, Brown RJ (2015) Partial and generalized lipodystrophy: comparison of baseline characteristics and response to metreleptin. J Clin Endocrinol Metab 100(5):1802–1810

Ding L, Li J, Song B, Xiao XU, Zhang B, Qi M, Huang W, Yang L, Wang Z (2016) Curcumin rescues high fat diet-induced obesity and insulin sensitivity in mice through regulating SREBP pathway. Toxicol Appl Pharmacol 304:99–109

Douglass JD, Dorfman MD, Thaler JP (2017) Glia: silent partners in energy homeostasis and obesity pathogenesis. Diabetologia 60(2):226–236

Duffy KB, Spangler EL, Devan BD, Guo Z, Bowker JL, Janas AM, Hagepanos A, Minor RK, DeCabo R, Mouton PR, Shukitt-Hale B (2008) A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. Neurobiol Aging 29(11):1680–1689

Dye RV, Miller KJ, Singer EJ, Levine AJ (2012) Hormone replacement therapy and risk for neurodegenerative diseases. Int J Alzheimer's Dis 2012:258454

Ebling FJ, Lewis JE (2018) Tanycytes and hypothalamic control of energy metabolism. Glia 66(6): 1176–1184

Elsakka M, Grigorescu E, Stănescu U, Dorneanu V (1990) New data referring to chemistry of Withania somnifera species. Rev Med Chir Soc Med Nat Iasi 94(2):385–387

Erta M, Quintana A, Hidalgo J (2012) Interleukin-6, a major cytokine in the central nervous system. Int J Biol Sci 8(9):1254

Eun CS, Lim JS, Lee J, Lee SP, Yang SA (2017) The protective effect of fermented Curcuma longa L. on memory dysfunction in oxidative stress-induced C6 gliomal cells, proinflammatory-activated BV2 microglial cells, and scopolamine-induced amnesia model in mice. BMC Complem Altern Med 17(1):1–2

Friedman JM, Mantzoros CS (2015) 20 years of leptin: from the discovery of the leptin gene to leptin in our therapeutic armamentarium. Metab Clin Exp 64(1):1–4

Fuente-Martín E, García-Cáceres C, Granado M, de Ceballos ML, Sánchez-Garrido MÁ, Sarman B, Liu ZW, Dietrich MO, Tena-Sempere M, Argente-Arizón P, Díaz F (2012) Leptin regulates glutamate and glucose transporters in hypothalamic astrocytes. J Clin Invest 122(11): 3900–3913

Gao Y, Ottaway N, Schriever SC, Legutko B, García-Cáceres C, de la Fuente E, Mergen C, Bour S, Thaler JP, Seeley RJ, Filosa J (2014) Hormones and diet, but not body weight, control hypothalamic microglial activity. Glia 62(1):17–25

García-Cáceres C, Fuente-Martín E, Burgos-Ramos E, Granado M, Frago LM, Barrios V, Horvath T, Argente J, Chowen JA (2011) Differential acute and chronic effects of leptin on hypothalamic astrocyte morphology and synaptic protein levels. Endocrinol 152(5):1809–1818

García-Cáceres C, Yi CX, Tschöp MH (2013) Hypothalamic astrocytes in obesity. Endocrinol Metab Clin 42(1):57–66
García-Cáceres C, Balland E, Prevot V, Luquet S, Woods SC, Koch M, Horvath TL, Yi CX, Chowen JA, Verkhratsky A, Araque A (2019) Role of astrocytes, microglia, and tanycytes in brain control of systemic metabolism. Nat Neurosci 22(1):7–14
Gavrilova V, Kajdzanoska M, Gjamovski V, Stefova M (2011) Separation, characterization and quantification of phenolic compounds in blueberries and red and black currants by HPLC− DAD− ESI-MS n. J Agr Food Chem 59(8):4009–4018
Gerhart DZ, Enerson BE, Zhdankina OY, Leino RL, Drewes LR (1998) Expression of the monocarboxylate transporter MCT2 by rat brain glia. Glia 22(3):272–281
Ghasemi F, Shafiee M, Banikazemi Z, Pourhanifeh MH, Khanbabaei H, Shamshirian A, Moghadam SA, ArefNezhad R, Sahebkar A, Avan A, Mirzaei H (2019) Curcumin inhibits NF-kB and Wnt/β-catenin pathways in cervical cancer cells. Pathol Res Pract 215(10):152556
Gokce EC, Kahveci R, Atanur OM, Gürer B, Aksoy N, Gokce A, Sargon MF, Cemil B, Erdogan B, Kahveci O (2015) Neuroprotective effects of Ganoderma lucidum polysaccharides against traumatic spinal cord injury in rats. Injury 46(11):2146–2155
Goodman T, Hajihosseini MK (2015) Hypothalamic tanycytes—masters and servants of metabolic, neuroendocrine, and neurogenic functions. Front Neurosci 9:387
Gunne LM, Andrén PE (1993) An animal model for coexisting tardive dyskinesia and tardive parkinsonism: a glutamate hypothesis for tardive dyskinesia. Clin Neuropharmacol 16:90–95
Gupta M, Kaur G (2016) Aqueous extract from the Withania somnifera leaves as a potential anti-neuroinflammatory agent: a mechanistic study. J Neuroinflamm 13(1):1–7
Gupta M, Kaur G (2018) Withania somnifera as a potential anxiolytic and anti-inflammatory candidate against systemic lipopolysaccharide-induced neuroinflammation. Neuromol Med 20(3):343–362
Gupta M, Kaur G (2019) Withania somnifera (L.) Dunal ameliorates neurodegeneration and cognitive impairments associated with systemic inflammation. BMC Complem Altern Med 19(1):1–8
Harrison L, Pfuhlmann K, Schriever SC, Pfluger PT (2019) Profound weight loss induces reactive astrogliosis in the arcuate nucleus of obese mice. Mol Metab 24:149–155
Hesari A, Ghasemi F, Salarinia R, Biglari H, Tabar Molla Hassan A, Abdoli V, Mirzaei H (2018) Effects of curcumin on NF-κB, AP-1, and Wnt/β-catenin signaling pathway in hepatitis B virus infection. J Cell Biochem 119(10):7898–7904
Hiramatsu K, Tsuneyoshi T, Ogawa T, Morihara N (2016) Aged garlic extract enhances heme oxygenase-1 and glutamate-cysteine ligase modifier subunit expression via the nuclear factor erythroid 2–related factor 2–antioxidant response element signaling pathway in human endothelial cells. Nutr Res 36(2):143–149
Hosseini A, Hosseinzadeh H (2018) Antidotal or protective effects of Curcuma longa (turmeric) and its active ingredient, curcumin, against natural and chemical toxicities: a review. Biomed Pharmacother 99:411–421
Hsuchou H, Pan W, Barnes MJ, Kastin AJ (2009) Leptin receptor mRNA in rat brain astrocytes. Peptides 30(12):2275–2280
Ide N, Lau BH (1999) Aged garlic extract attenuates intracellular oxidative stress. Phytomed 6(2): 125–131
Jahfar M (2003) Glycosyl composition of polysaccharide from Tinospora cordifolia. Acta pharmaceutica (Zagreb, Croatia) 53(1):65–69
Jana N, Basu A, Tandon PN (eds) (2016) Infection: The common link in brain pathology, 1st edn. Springer, Singapore
Jayaram B, Pan W, Wang Y, Hsuchou H, Mace A, Cornelissen-Guillaume GG, Mishra PK, Koza RA, Kastin AJ (2013) Astrocytic leptin-receptor knockout mice show partial rescue of leptin resistance in diet-induced obesity. J Appl Physiol 114(6):734–741

Jeyasri R, Muthuramalingam P, Suba V, Ramesh M, Chen JT (2020) Bacopa monnieri and their bioactive compounds inferred multi-target treatment strategy for neurological diseases: a cheminformatics and system pharmacology approach. Biomol 10(4):536

John J (2014) Therapeutic potential of Withania somnifera: a report on phyto-pharmacological properties. Int J Pharma Sci Res 5(6):2131–2148

Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, Bickford PC (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci 19(18): 8114–8121

Joseph JA, Arendash G, Gordon M, Diamond D, Shukitt-Hale B, Morgan DA, Denisova NA (2003) Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. Nutr Neurosci 6(3):153–162

Kaur J, Debnath J (2015) Autophagy at the crossroads of catabolism and anabolism. Nat Rev Mol Cell Biol 16(8):461–472

Kaur H, Patro I, Tikoo K, Sandhir R (2015) Curcumin attenuates inflammatory response and cognitive deficits in experimental model of chronic epilepsy. Neurochem Int 89:40–50

Khatian N, Aslam M (2019) Effect of Ganoderma lucidum on memory and learning in mice. Clin Phytosci 5(1):1–8

Kim JG, Suyama S, Koch M, Jin S, Argente-Arizon P, Argente J, Liu ZW, Zimmer MR, Jeong JK, Szigeti-Buck K, Gao Y (2014) Leptin signaling in astrocytes regulates hypothalamic neuronal circuits and feeding. Nat Neurosci 17(7):908–910

Konar A, Shah N, Singh R, Saxena N, Kaul SC, Wadhwa R, Thakur MK (2011) Protective role of Ashwagandha leaf extract and its component withanone on scopolamine-induced changes in the brain and brain-derived cells. PloS one 6(11):e27265

Konar A, Gautam A, Thakur MK (2015) Bacopa monniera (CDRI-08) upregulates the expression of neuronal and glial plasticity markers in the brain of scopolamine induced amnesic mice. Evid Based Complemt Alternat Med 2015:837012

Kongkeaw C, Dilokthornsakul P, Thanarangsarit P, Limpeanchob N, Scholfield CN (2014) Meta-analysis of randomized controlled trials on cognitive effects of Bacopa monnieri extract. J Ethnopharmacol 151(1):528–535

Kosaraju J, Chinni S, Roy PD, Kannan E, Antony AS, Kumar MS (2014) Neuroprotective effect of Tinospora cordifolia ethanol extract on 6-hydroxy dopamine induced Parkinsonism. Indian J Pharmacol 46(2):176

Kuboyama T, Tohda C, Komatsu K (2006) Withanoside IV and its active metabolite, sominone, attenuate Aβ (25–35) -induced neurodegeneration. Eur J Neurosci 23(6):1417–1426

Kuboyama T, Tohda C, Komatsu K (2014) Effects of Ashwagandha (roots of Withania somnifera) on neurodegenerative diseases. Biol Pharma Bull 37(6):892–897

Kulkarni SK, Dhir A (2008) Withania somnifera: an Indian ginseng. Prog Neuro-psychopharmacol Biol Psychiatr 32(5):1093–1105

Lai CS, Yu MS, Yuen WH, So KF, Zee SY, Chang RC (2008) Antagonizing β-amyloid peptide neurotoxicity of the anti-aging fungus Ganoderma lucidum. Brain Res 1190:215–224

Lam TK, Gutierrez-Juarez R, Pocai A, Rossetti L (2005) Regulation of blood glucose by hypothalamic pyruvate metabolism. Science 309(5736):943–947

Lee WH, Loo CY, Bebawy M, Luk F, Mason RS, Rohanizadeh R (2013) Curcumin and its derivatives: their application in neuropharmacology and neuroscience in the 21st century. Curr Neuropharmacol 11(4):338–378

Leung HW, Foo G, Banumurthy G, Chai X, Ghosh S, Mitra-Ganguli T, VanDongen AM (2017) The effect of Bacopa monnieri on gene expression levels in SH-SY5Y human neuroblastoma cells. Plos One 12(8):e0182984

Li R, Huang YG, Fang D, Le WD (2004) Epigallocatechin gallate inhibits lipopolysaccharide-induced microglial activation and protects against inflammation-mediated dopaminergic neuronal injury. J Neurosci Res 78(5):723–731

Mariani MM, Kielian T (2009) Microglia in infectious diseases of the central nervous system. J Neuroimmune Pharmacol 4(4):448–461
Mathew M, Subramanian S (2012) Evaluation of the anti-amyloidogenic potential of nootropic herbal extracts in vitro. Int J Pharma Sci Res 3(11):4276
Matias I, Morgado J, Gomes FC (2019) Astrocyte heterogeneity: impact to brain aging and disease. Front Aging Neurosci 11:59
McDougal DH, Hermann GE, Rogers RC (2013) Astrocytes in the nucleus of the solitary tract are activated by low glucose or glucoprivation: evidence for glial involvement in glucose homeostasis. Front Neurosci 7:249
McGuire SO, Sortwell CE, Shukitt-Hale B, Joseph JA, Hejna MJ, Collier TJ (2006) Dietary supplementation with blueberry extract improves survival of transplanted dopamine neurons. Nutr Neurosci 9(5-6):251–258
Menon I, Spudich A (2010) The Ashtavaidya physicians of Kerala: a tradition in transition. J Ayurveda Int Med 1(4):245
Mishra R, Kaur G (2013) Aqueous ethanolic extract of Tinospora cordifolia as a potential candidate for differentiation-based therapy of glioblastomas. PLoS One 8(10):e78764
Mishra R, Manchanda S, Gupta M, Kaur T, Saini V, Sharma A, Kaur G (2016) Tinospora cordifolia ameliorates anxiety-like behavior and improves cognitive functions in acute sleep deprived rats. Sci Rep 6(1):1–5
Mizushina Y, Hanashima L, Yamaguchi T, Takemura M, Sugawara F, Saneyoshi M, Matsukage A, Yoshida S, Sakaguchi K (1998) A mushroom fruiting body-inducing substance inhibits activities of replicative DNA polymerases. Biochem Biophys Res Commun:17–22
Momtazi AA, Derosa G, Maffioli P, Banach M, Sahebkar A (2016) Role of microRNAs in the therapeutic effects of curcumin in non-cancer diseases. Mol Diagn Ther 20(4):335–345
Moraes WA (2015) Current pharmacological and non-pharmacological therapies for neurodegenerative diseases. Young Persp Old Dis:23–48
Morgello S, Uson RR, Schwartz EJ, Haber RS (1995) The human blood-brain barrier glucose transporter (GLUT1) is a glucose transporter of gray matter astrocytes. Glia 14(1):43–54
Morihara N, Hayama M, Fujii H (2011) Aged garlic extract scavenges superoxide radicals. Plant Foods Hum Nutr 66(1):17–21
Morrish P (2012) Prescribing in Parkinson's disease: a story of hope and adverse events. Prac Neurol 12(5):335–340
Mukherjee D, Banerjee S (2013) Learning and memory promoting effects of crude garlic extract
Mukherjee S, Fried A, Hussaini R, White R, Baidoo J, Yalamanchi S, Banerjee P (2018) Phytosomal curcumin causes natural killer cell-dependent repolarization of glioblastoma (GBM) tumor-associated microglia/macrophages and elimination of GBM and GBM stem cells. J Exp Clin Can Res 37(1):1–8
Mutalik M, Mutalik M (2011) Tinospora cordifolia and its varied activities: what is believed and what is known. Int J Curr Res Rev 3:94
Naik AA, Patro IK, Patro N (2015) Slow physical growth, delayed reflex ontogeny, and permanent behavioral as well as cognitive impairments in rats following intra-generational protein malnutrition. Front Neurosci 9:446
Naik AA, Patro N, Seth P, Patro IK (2017) Intra-generational protein malnutrition impairs temporal astrogenesis in rat brain. Biol Open 6(7):931–942
Nakajima Y, Iguchi H, Kamisuki S, Sugawara F, Furuichi T, Shinoda Y (2016) Low doses of the mycotoxin citrinin protect cortical neurons against glutamate-induced excitotoxicity. J Toxicol Sci 41(2):311–319
Nemetchek MD, Stierle AA, Stierle DB, Lurie DI (2017) The ayurvedic plant Bacopa monnieri inhibits inflammatory pathways in the brain. J Ethnopharmacol 197:92–100
Nillert N, Pannangrong W, Welbat JU, Chaijaroonkhanarak W, Sripanidkulchai K, Sripanidkulchai B (2017) Neuroprotective effects of aged garlic extract on cognitive dysfunction and neuroinflammation induced by β-amyloid in rats. Nutrients 9(1):24

Pabon MM, Jernberg JN, Morganti J, Contreras J, Hudson CE, Klein RL, Bickford PC (2012) A spirulina-enhanced diet provides neuroprotection in an α-synuclein model of Parkinson's disease

Pan W, Hsuchou H, Jayaram B, Khan RS, Huang EY, Wu X, Chen C, Kastin AJ (2012) Leptin action on nonneuronal cells in the CNS: potential clinical applications. Ann New York Aca Sci 1264(1):64

Pandey V, Vaishya JK, Balakrishnan P, Nesari TM (2020) Nutritional aspects of Tinospora cordifolia (Giloe). Med Plants-Int J Phytomed Relat Indus 12(1):158–160

Papandreou MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Klimis-Zacas D, Margarity M, Lamari FN (2009) Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behav Brain Res 198(2): 352–358

Parada E, Buendia I, Navarro E, Avendaño C, Egea J, López MG (2015) Microglial HO-1 induction by curcumin provides antioxidant, antineuroinflammatory, and glioprotective effects. Mol Nutr Food Res 59(9):1690–1700

Patro N, Sharma A, Kariaya K, Patro I (2011) Spirulina platensis protects neurons via suppression of glial activation and peripheral sensitization leading to restoration of motor function in collagen-induced arthritic rats.

Patro N, Naik AA, Patro IK (2019) Developmental changes in oligodendrocyte genesis, myelination, and associated behavioral dysfunction in a rat model of intra-generational protein malnutrition. Mol Neurobiol 56(1):595–610

Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL (2004) Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science 304(5667):110–115

Prakash R, Sandhya E, Ramya N, Dhivya R, Priyadarshini M, Sakthi Priya B (2017) Neuroprotective activity of ethanolic extract of Tinospora cordifolia on LPS induced neuroinflammation. Transl Biomed 8(4):135

Rai M, Jogee PS, Agarkar G, Santos CA (2016) Anticancer activities of Withania somnifera: current research, formulations, and future perspectives. Pharm Biol 54(2):189–197

Ramachandran C, Quirin KW, Escalon E, Melnick SJ (2014) Improved neuroprotective effects by combining Bacopa monnieri and Rosmarinus officinalis supercritical CO2 extracts. J Evi Based Comp Altern Med 19(2):119–127

Rawal AK, Muddeshwar MG, Biswas SK (2004) Rubia cordifolia, Fagonia cretica linn and Tinospora cordifolia exert neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation. BMC Comp Altern Med 4(1):1–9

Ren ZL, Wang CD, Wang T, Ding H, Zhou M, Yang N, Liu YY, Chan P (2019) Ganoderma lucidum extract ameliorates MPTP-induced parkinsonism and protects dopaminergic neurons from oxidative stress via regulating mitochondrial function, autophagy, and apoptosis. Acta Pharmacologica Sinica 40(4):441–450

Robb JL, Morrissey NA, Potter PG, Smithers HE, Beall C, Ellacott KL (2020) Immunometabolic changes in Glia–A potential role in the pathophysiology of obesity and diabetes. Neurosci 447: 167–181

Roch-Torreilles I, Camu W, Hillaire-Buys D (2000) Adverse efects of riluzole (Rilutek) in the treatment of amyotrophic lateral sclerosis. Therapie 55(2):303–312

Rodríguez EM, Blázquez JL, Pastor FE, Peláez B, Pena P, Peruzzo B, Amat P (2005) Hypothalamic tanycytes: a key component of brain–endocrine interaction. Int Rev Cytol 247:89–164

Rottkamp DM, Rudenko IA, Maier MT, Roshanbin S, Yulyaningsih E, Perez L, Valdearcos M, Chua S, Koliwad SK, Xu AW (2015) Leptin potentiates astrogenesis in the developing hypothalamus. Mol Metab 4(11):881–889

Saha S, Ghosh S (2012) Tinospora cordifolia: one plant, many roles. Anc Sci Life 31(4):151

Schur EA, Melhorn SJ, Oh SK, Lacy JM, Berkseth KE, Guyenet SJ, Sonnen JA, Tyagi V, Rosalynn M, De Leon B, Webb MF (2015) Radiologic evidence that hypothalamic gliosis is associated with obesity and insulin resistance in humans. Obesity 23(11):2142–2148

Shah N, Kataria H, Kaul SC, Ishii T, Kaur G, Wadhwa R (2009) Effect of the alcoholic extract of Ashwagandha leaves and its components on proliferation, migration, and differentiation of glioblastoma cells: combinational approach for enhanced differentiation. Cancer Sci 100(9): 1740–1747

Shah N, Singh R, Sarangi U, Saxena N, Chaudhary A, Kaur G, Kaul SC, Wadhwa R (2015) Combinations of Ashwagandha leaf extracts protect brain-derived cells against oxidative stress and induce differentiation. PLoS One 10(3):e0120554

Sharma A, Kaur G (2018) Tinospora cordifolia as a potential neuroregenerative candidate against glutamate induced excitotoxicity: an in vitro perspective. BMC Comp Altern Med 18(1):1–7

Sharma P, Dwivedee BP, Bisht D, Dash AK, Kumar D (2019) The chemical constituents and diverse pharmacological importance of Tinospora cordifolia. Heliyon 5(9):e02437

Sharma A, Bajaj P, Bhandari A, Kaur G (2020) From ayurvedic folk medicine to preclinical neurotherapeutic role of a miraculous herb. Tinospora cordifolia. Neurochemistry Int 141: 104891

Shinomol GK (2011) Bacopa monnieri modulates endogenous cytoplasmic and mitochondrial oxidative markers in prepubertal mice brain. Phytomed 18(4):317–326

Shirolkar A, Sharma B, Lata S, Dabur R (2016) Guduchi Sawras (Tinospora cordifolia): an Ayurvedic drug treatment modulates the impaired lipid metabolism in alcoholics through dopaminergic neurotransmission and anti-oxidant defense system. Biomed Pharmacother 83: 1265–1277

Singh RH, Udupa KN (1993) Clinical and experimental studies on rasayana drugs and rasayana therapy. Special Research Monograph, Central Council for Research in Ayurveda and Siddha (CCRAS), Ministry of Health and Family Welfare, New Delhi

Singh N, Bhalla M, de Jager P, Gilca M (2011) An overview on ashwagandha: a Rasayana (rejuvenator) of Ayurveda. Afr J Trad Comp Altern Med 8(5S)

Sinha S, Patro N, Patro IK (2018) Maternal protein malnutrition: current and future perspectives of spirulina supplementation in neuroprotection. Front Neurosci 12:966

Sinha S, Patro N, Patro IK (2020a) Amelioration of neurobehavioral and cognitive abilities of F1 progeny following dietary supplementation with Spirulina to protein malnourished mothers. Brain Behav Immun 85:69–87

Sinha S, Patro N, Tiwari PK, Patro IK (2020b) Maternal Spirulina supplementation during pregnancy and lactation partially prevents oxidative stress, glial activation and neuronal damage in protein malnourished F1 progeny. Neurochem Int 141:104877

Song H, Lu Y, Qu Z, Mossine VV, Martin MB, Hou J, Cui J, Peculis BA, Mawhinney TP, Cheng J, Greenlief CM (2016) Effects of aged garlic extract and FruArg on gene expression and signaling pathways in lipopolysaccharide-activated microglial cells. Sci Rep 6(1):1

Sorrenti V, Contarini G, Sut S, Dall'Acqua S, Confortin F, Pagetta A, Giusti P, Zusso M (2018) Curcumin prevents acute neuroinflammation and long-term memory impairment induced by systemic lipopolysaccharide in mice. Front Pharmacol 9:183

Stough C, Scholey A, Cropley V, Wesnes K, Zangara A, Pase M, Savage K, Nolidin K, Downey L (2013) Examining the cognitive effects of a special extract of Bacopa monniera (CDRI08: Keenmnd): a review of ten years of research at Swinburne University. J Pharm Sci 16(2): 254–258

Strömberg I, Gemma C, Vila J, Bickford PC (2005) Blueberry-and spirulina-enriched diets enhance striatal dopamine recovery and induce a rapid, transient microglia activation after injury of the rat nigrostriatal dopamine system. Exp Neurol 196(2):298–307

Subash S, Essa MM, Al-Adawi S, Memon MA, Manivasagam T, Akbar M (2014) Neuroprotective effects of berry fruits on neurodegenerative diseases. Neural Regen Res 9(16):1557

Sweeney MI, Kalt W, MacKinnon SL, Ashby J, Gottschall-Pass KT (2002) Feeding rats diets enriched in lowbush blueberries for six weeks decreases ischemia-induced brain damage. Nutr Neurosci 5(6):427–431

Tanaka J, Toku K, Matsuda S, Sudo S, Fujita H, Sakanaka M, Maeda N (1998) Induction of resting microglia in culture medium devoid of glycine and serine. Glia 24(2):198–215

Thomson M, Al-Qattan KK, Divya JS, Ali M (2015) Anti-diabetic and anti-oxidant potential of aged garlic extract (AGE) in streptozotocin-induced diabetic rats. BMC Comp Altern Med 16(1):1–9

Trivedi MK, Gangwar M, Mondal SC, Jana S (2017) Immunomodulatory properties and biomarkers characterization of novel Withania somnifera based formulation supplemented with minerals in Sprague Dawley rats. Orient Pharm Exp Med 17(1):59–69

Vatier C, Fetita S, Boudou P, Tchankou C, Deville L, Riveline JP, Young J, Mathivon L, Travert F, Morin D, Cahen J (2016) One-year metreleptin improves insulin secretion in patients with diabetes linked to genetic lipodystrophic syndromes. Diabetes Obes Metab 18(7):693–697

Verkhratsky A, Nedergaard M (2014) Astroglial cradle in the life of the synapse. Philos Trans R Soc B Bio Sci 369(1654):20130595

Verkhratsky A, Nedergaard M (2016) The homeostatic astroglia emerges from evolutionary specialization of neural cells. Philosop Trans Royal Soc B Bio Sci 371(1700):20150428

Verkhratsky A, Nedergaard M (2018) Physiology of astroglia. Physiol Rev 98(1):239–389

Verkhratsky A, Parpura V, Pekna M, Pekny M, Sofroniew M (2014) Glia in the pathogenesis of neurodegenerative diseases. Biochem Soc Trans 42(5):1291–1301

Wang Y, Chang CF, Chou J, Chen HL, Deng X, Harvey BK, Cadet JL, Bickford PC (2005) Dietary supplementation with blueberries, spinach, or spirulina reduces ischemic brain damage. Exp Neurol 193(1):75–84

Wang L, Pavlou S, Du X, Bhuckory M, Xu H, Chen M (2019) Glucose transporter 1 critically controls microglial activation through facilitating glycolysis. Mol Neurodegen 14(1):1–5

Williams RJ, Mohanakumar KP, Beart PM (2016) Neuro-nutraceuticals: Further insights into their promise for brain health. Neurochem Int 95:1–3

Willis LM, Freeman L, Bickford PC, Quintero EM, Umphlet CD, Moore AB, Goetzl L, Granholm AC (2010) Blueberry supplementation attenuates microglial activation in hippocampal intraocular grafts to aged hosts. Glia 58(6):679–690

Wolf SA, Boddeke HW, Kettenmann H (2017) Microglia in physiology and disease. Annu Rev Physiol 79:619–643

Yang L, Qi Y, Yang Y (2015) Astrocytes control food intake by inhibiting AGRP neuron activity via adenosine A1 receptors. Cell Rep 11(5):798–807

Yi CX, Habegger KM, Chowen JA, Stern J, Tschöp MH (2011) A role for astrocytes in the central control of metabolism. Neuroendocrinol 93(3):143–149

You Q, Wang B, Chen F, Huang Z, Wang X, Luo PG (2011) Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. Food Chem 125(1):201–208

Young AB (2009) Four decades of neurodegenerative disease research: how far we have come! J Neurosci 29(41):12,722–12,728

Yu Y, Shen Q, Lai Y, Park SY, Ou X, Lin D, Jin M, Zhang W (2018) Anti-inflammatory effects of curcumin in microglial cells. Front Pharmacol 9:386

Zhang R, Xu S, Cai Y, Zhou M, Zuo X, Chan P (2011) Ganoderma lucidum protects dopaminergic neuron degeneration through inhibition of microglial activation. Evid Based Complement Alternat Med 2011:156810

Zhang X, Dong H, Zhang S, Lu S, Sun J, Qian Y (2015) Enhancement of LPS-induced microglial inflammation response via TLR4 under high glucose conditions. Cell Physiol Biochem 35(4): 1571–1581

Zhang Y, Reichel JM, Han C, Zuniga-Hertz JP, Cai D (2017) Astrocytic process plasticity and IKKβ/NF-κB in central control of blood glucose, blood pressure, and body weight. Cell Metab 25(5):1091–1102

Zhao J, Nakamura N, Hattori M, Kuboyama T, Tohda C, Komatsu K (2002) Withanolide derivatives from the roots of Withania somnifera and their neurite outgrowth activities. Chem Pharm Bull 50(6):760–765

Zhao HB, Lin SQ, Liu JH, Lin ZB (2004) Polysaccharide extract isolated from Ganoderma lucidum protects rat cerebral cortical neurons from hypoxia/reoxygenation injury. J Pharm Sci 95(2): 294–298

Zhou H, Qu Z, Mossine VV, Nknolise DL, Li J, Chen Z, Cheng J, Greenlief CM, Mawhinney TP, Brown PN, Fritsche KL (2014) Proteomic analysis of the effects of aged garlic extract and its FruArg component on lipopolysaccharide-induced neuroinflammatory response in microglial cells. PLoS One 9(11):e113531

Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B (1996) Studies on the immunomodulatory effects of Ashwagandha. J Ethnopharmacol 50(2):69–76

The Role of Glia in Huntington's Disease

Tripti Joshi and Nihar Ranjan Jana

Abstract

The accumulation of intracellular protein deposits as inclusion bodies is the common pathological hallmark of most age-related neurodegenerative disorders including Huntington's diseases (HD). HD is an autosomal dominantly inherited neurodegenerative disorder caused by an aberrant expansion of CAG repeats in the coding region of huntingtin gene. The mutant huntingtin protein form aggregates in neurons and causes neuronal dysfunction and degeneration in multiple ways including transcriptional dysregulation, dysfunction in protein quality control system, etc. Marked inflammatory reaction has been observed in postmortem brain samples of HD patients as well as in various mouse models of HD that could be linked at least in part with the pathogenesis of HD. Here we review the recent studies that have revealed the critical role of glial cells and inflammation in the pathogenesis of HD.

Keywords

Huntington's disease · Excitotoxicity · Neuroinflammation · Microglia · Astroglia

T. Joshi
Cellular and Molecular Neuroscience Laboratory, National Brain Research Centre, Manesar, Gurgaon, Haryana, India

N. R. Jana (✉)
School of Bioscience, Indian Institute of Technology, Kharagpur, Kharagpur, West Bengal, India
e-mail: nihar@iitkgp.ac.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_24

1 Introduction

Neuroinflammation is a process which occurs in response to a variety of damage signals that include injury, infection, trauma, recognition of novel substance (Benarroch 2013), or aggregation of misfolded proteins in most age-related neurodegenerative disorders among many more. The principal mediators of this process are microglia and astrocytes that release chemokines, cytokines, neuromodulators, and various other effector molecules as a triggering signal of alarm and defense. Neuroinflammation can be regarded as an alarm for CNS to robustly defend itself from the potential threat although chronic activity of this system is detrimental to the neuron.

Astrocytes and microglia serve as dynamic surveillance system and maintain optimal microenvironment for the neuronal function in the absence of any damage signal. They regulate synaptic communication and are involved in synaptic pruning (Kettenmann et al. 2013). They are responsive to various neurotransmitters and in turn affect the neuronal function by a plethora of chemical secretions and neuromodulators (Pekny et al. 2016). Resting or more suitably called "surveying" microglia play an important role in the cellular and synaptic homeostasis. Neuroprotective effects of microglia involve microglial production of growth factors (Boscia et al. 2009) and increased uptake of glutamate (Persson and Ronnback 2012). Microglia also exhibits pro-inflammatory and cytotoxic function when activated. Depending upon the activation pattern, microglia is categorized as M1 or pro-inflammatory and M2 or anti-inflammatory subtypes (Benarroch 2013). Previously considered as only a passive cell type, astrocytes are of great importance as they protect the neuron from excess glutamate that can result in glutamate-mediated excitotoxicity (Belanger and Magistretti 2009). Upon activation, astrocytes undergo context-dependent reactive astrogliosis that can be either protective or harmful (Palpagama et al. 2019).

In neurodegenerative diseases like Huntington's disease (HD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS), debris of dead neurons are thought to activate glial-mediated inflammatory response (Joshi et al. 2019). This unchecked chronic process leads to extensive death of neurons leading to further neurotoxicity. Without intervention, this process establishes itself as a vicious cycle that results in rapid progression of the disease. HD is a progressive neurodegenerative disorder inherited in an autosomal dominant manner. It is characterized by abnormal increase of CAG repeats in the *Huntingtin* (*HTT*) gene that translates to form mutant huntingtin (mHTT), which eventually cleaves, and N-terminal portion of mHTT containing polyglutamine repeat form aggregates ubiquitously in neurons and glial cells. These aggregates interfere with the normal functioning of various cellular proteins like important molecular chaperones and proteasome components by interacting with them and rendering them unavailable for their routine functions, hence leaving a proteostasis imbalance (Li et al. 2003). Mutant HTT also aberrantly interacts with many nuclear proteins including transcription factors/coactivators and leads to global transcriptional alterations (van Hagen et al. 2017; Chen et al. 2018; Hodges et al. 2006; Olzscha et al. 2011). Selective loss of striatal medium spiny

neurons (MSNs) is very peculiar to this disease that can be attributed to the glutamate excitotoxicity theory (Calabresi et al. 1998) as these MSNs are largely innervated by glutamatergic axons from the cortical neurons. Cortical areas and other brain regions like the hippocampus (Giralt et al. 2011) are also affected during the course of progression of the disease. HD patients show motor dysfunction characterized by "'chorea" (Gusella et al. 1983), psychiatric disturbances (Imarisio et al. 2008), and atrophied striatum (Reiner et al. 1988). The number of CAG repeats present in the mHTT protein is the driving force for the onset of the disease. The most pronounced effect of this is seen in juvenile HD where longest repeats are observed (Mangiarini et al. 1996). Repeat length of over 35 CAGs increases the risk of the disease (Vonsattel and DiFiglia 1998). Although neuroinflammation encompasses various glial cells like microglia, astrocytes, oligodendrocytes, and other peripheral inflammation mediating cells like macrophages, for this chapter, we have focused on the two major glial cell types of the brain: microglia and astrocytes. Existing literature exposes the role of microglia and astrocytes in HD pathophysiology, but more detailed experiments are required to clearly dissect the role of mHTT in neuroinflammation and how it sets the threshold for neuroprotective or neurotoxic cascade events.

2 Microglial Activation and Reactive Astrogliosis: Key Processes in Neuroinflammation

Microglia and astrocytes are the key players in neuroinflammation in a plethora of diseases including neurodegenerative disorders. What makes them special is the dual nature of neuroprotection and neurotoxicity. In a healthy brain, they are an invaluable source of defense mechanism, but chronic activation of neuroinflammatory components proves detrimental to the neuron.

Microglial activation: Microglia are the resident immune cells of the brain, making approximately 10% of the total cell population. "Resting" and "activated" prefixes in microglial activation are now considered as an oversimplification of their actual functional contribution (Benarroch 2013; Hanisch and Kettenmann 2007; Kettenmann et al. 2013). "Surveying" and "effector" terms are thought to rightly describe the previously resting and activated terminology, respectively (Benarroch 2013). Microglia dynamically survey their defined space (Nimmerjahn et al. 2005), and upon exposure to any damage signal, they get converted to activated or effector state. The regulation of these two phenotypes depend upon various ion channels, cell adhesion molecules, and receptors (U.-K. Hanisch 2013) present on the surface of microglia that act as sensors for the signal presented by its microenvironment.

Morphologically, the surveying microglia present a ramified shape characterized by numerous branching like projections from the small soma. Time lapse imaging of surveying microglia shown in an elegant experiment by Nimmerjahn et al. (Nimmerjahn et al. 2005) clearly demonstrated the de novo formation and retraction of microglial processes in minutes timescale. To maintain the surveillance state of microglial cells, a number of constitutive "off" signals (Ransohoff and Cardona

2010) are required. One of these is the contact-dependent interaction between neuronal CD200 and microglial CD200 (Benarroch 2013). The other off signal in the healthy brain is the constitutive expression of CX3CL1 (fractalkine) on neurons and its partner receptor CX3CR1 on microglia.

In vitro studies have shown a large number of molecular changes occurring inside and at the surface of the microglia during the process of activation. Purinergic P2X receptors, a ligand gated Ca^{+2} channel, are involved in Ca^{+2} signaling critical for microglial function (Benarroch 2013). Also, chloride channels translocate from cytosol to plasma membrane to promote release of reactive oxygen species (ROS) during microglial activation. The "on" signals trigger the activation of microglia (Biber et al. 2007). These signals include microbial, viral, and fungal challenges. Also, misfolded proteins, externalization of phosphatidylserine, and complement proteins trigger the microglial activation. Morphological changes also occur during activation where the projection becomes shorter and microglia no longer represent a ramified structure. Microglial activation produces varying cell responses that are broadly categorized into M1 and M2 functional states. The M1 state occurs when activation of the transcription factor NF-κB leads to the production of the pro-inflammatory cytokines (IL-1β, IL-6, IL-23, and TNF-α) and cytotoxic molecules (ROS, NO). Surface expression of MHC proteins also occurs in microglia during activation. Components of complement system also confer M1 microglial state. M2 functional adaptation is anti-inflammatory in function as it is involved in the production and secretion of IL-10, IL-4, IL-13, and TGFβ which are neuroprotective in nature (Mosser and Edwards 2008; Benarroch 2013; Palpagama et al. 2019). It is reported that M1 and M2 states are interconvertible and is regulated by nuclear receptor peroxisome proliferator-activated receptor-γ (Mandrekar-Colucci et al. 2012). Microglia behaves as a double-edged sword (Biber et al. 2007) in the process of neuroinflammation. The neuroprotective and pro-inflammatory effect depends upon the type and duration of stimulus received by these cells and the functional state the microglia enter into. Depending upon the activation response, microglia behave differently in various pathological conditions.

Astroglial activation: Astrocytes, the most abundant cells, possess a "bushy" appearance in the healthy brain owing to the presence of six or so major branches and many fine branchlets. They help in maintaining brain homeostasis and are involved in synaptic function by regulating synapse formation and maturation, neurotransmitter homeostasis, and release of gliotransmitters; they also regulate pH, water, and ion homeostasis. A blood vessel-associated end foot allows them to form an integral part of the blood-brain barrier (Sofroniew and Vinters 2010); (MacVicar and Newman 2015) (Benraiss et al. 2016; Nedergaard et al. 2003; Pekny et al. 2016) (Verkhratsky and Nedergaard 2018). Each astrocyte caters to the need of the nearby neurons, hence forming discrete territories, and these non-overlapping functional anatomical domains are connected via gap junctions forming a well-balanced network (Halassa et al. 2007) (Oberheim et al. 2009) (Nedergaard et al. 2003) (Rouach et al. 2004). The astrocytic processes surround the synapses enabling them to sense, respond, and modulate their microenvironment (Belanger and Magistretti 2009). One of the main role of astrocytes is at the synapse where it

regulates the level of different neurotransmitters (Chung et al. 2015) (Blackburn et al. 2009; (Sofroniew and Vinters 2010). Astrocytes uptake the glutamate released into the synaptic cleft by glutaminergic neurons (Belanger and Magistretti 2009). This is an ATP-dependent process requiring one ATP per glutamate molecule uptake. Glutamate is then enzymatically converted to glutamine and is cycled back to the neuron, hence ensuring its availability to synthesize neurotransmitters again. Excitatory amino acid transporter (EAAT) in humans and glutamate transporter 1 (GLT1) in murine models are the receptors responsible for glutamate uptake (Rothstein et al. 1994).

Astrocytes along with microglia act in the process of neuroinflammation and can be neuroprotective as well as neurotoxic depending upon the stimulus and the signals from microglia. There is an extensive communication between neurons, microglia, and astrocytes that allows maintaining brain homeostasis. Astrocytes can get activated by a variety of signals through a process called "reactive astrogliosis" and result in the release of various cytokines, chemokines, and growth factors. Reactive astrogliosis is defined as a spectrum of changes including cellular morphology, molecular expression, and in severe cases scar formation (Sofroniew 2009). Glial fibrillary acidic protein (GFAP) has been identified as a reliable marker of reactive astrocytes, and the expression of GFAP is more pronounced in severe and moderate reactive astrogliosis as compared to normal healthy astrocytes (Sofroniew and Vinters 2010). Healthy astrocytes have defined functional boundaries such that their processes do not overlap, but this distinction is absent in severely reactive astrogliosis and glial scar (Sofroniew 2009; Sofroniew and Vinters 2010). Few studies show that the glial scar forming astrocytes isolate the region and prevent the entry of neuroinflammatory cells (Voskuhl et al. 2009) (Phatnani and Maniatis 2015).

In a set of elaborate experiments performed by Zamanian et al. (2012), they confirmed the two categories of reactive astrocytes as A1 and A2 induced by neuroinflammation and ischemia, respectively (Zamanian et al. 2012) (Liddelow et al. 2017). A1 astrocytes which could be considered analogous to the M1 activation state of microglia are pro-inflammatory, while A2 subtype was demonstrated to be neuroprotective as it upregulated the expression of neurotropic factors (Liddelow et al. 2017). They also suggested the role of microglia in the activation of the A1 astrocytes through the secretion of Il-1α and TNF in vivo and in vitro implicating its role in neurodegeneration by analyzing postmortem samples in AD, HD, ALS, etc.

3 Neuroinflammation in HD

Glial pathology has been reported in the brain of many HD mouse models (Shin et al. 2005) and in postmortem brains of HD patients (Sapp et al. 2001). The mHTT aggregates were also present in glial cells in the brain of HD mice and also HD patients (Bradford et al. 2010; Shin et al. 2005). In HD patients, astrocytes and microglia become activated, as shown by the upregulation of GFAP and thymosin

b4, respectively, and the degree of activation correlates with disease progression (Faideau et al. 2010; Sapp et al. 2001).

Role of microglia in HD inflammation: Neuroinflammation has been majorly attributed to microglia and its activation. Upon exposure to the damage signals as mentioned in microglial activation section, microglia transform from its surveillance state to an activated state and release pro-inflammatory cytokines like TNF-α and IL-1β which switch on the neuroinflammation cascade and may result in neuronal death. Postmortem HD brain study by Sapp and colleagues (Sapp et al. 2001) showed activated microglia in basal ganglia that are majorly affected in HD and frontal cortex. They reported a direct correlation between the progression of neuronal loss to the number of activated microglia present in striatum and cortex. They found grade 2 and 3 HD brains had more reactive microglia. A study by Tai and colleagues (Tai et al. 2007) reported for the first time the presence of in vivo microglial activation in HD presymptomatic gene carriers by PET analysis. Microglial activation was also reported in R6/2 mice (Simmons et al. 2007). The mHTT is expressed in microglia as well (Shin et al. 2005; Jansen et al. 2017) and could possibly be a direct neuroinflammatory inducer. Neuronal mHTT accumulation is also considered as a triggering signal for microglial activation (Kraft et al. 2012). Two major pathways have been majorly studied in HD (Palpagama et al. 2019): nuclear factor kappa B (NF-κB) and kynurenine pathway. NF-κB is an important pathway implicated in microglial activation and hence HD pathogenesis. mHTT interacts with one of the subunits of IKK complex and activates it (Khoshnan et al. 2004) leading to NF-κB dependent promotion of gene expression of pro-inflammatory cytokines. This mechanism is shown in PC12 cell culture as well as in the striatum and cortex of R6/2 HD mice (Palpagama et al. 2019). Another pathway implicated in HD pathogenesis is the kynurenine pathway. Studies by Guidetti et al. (Guidetti et al. 2004) showed increased level of neurotoxic kynurenine pathway metabolites quinolinic acid and 3-hydroxykynurenine (3HK) in low grade HD tissue and in R6/2 mice (Trager et al. 2014) (Khoshnan et al. 2004; Palpagama et al. 2019). In another set of very recent study by Crasper and colleagues, they eliminated 99% of microglia by using an inhibitor of colony-stimulating factor 1 receptor (CSF1Ri). This FDA-approved CSF1Ri pexidartinib (Crapser et al. 2020) was shown to ameliorate disease pathology by majorly reducing the atrophied striatal volume. A number of studies have shown potential of microglia to sense their environment and act accordingly. It is an important component of the dynamic mechanism of neuroinflammation and interacts with astrocytes and neurons.

Role of astrocytes in HD pathophysiology: The mHTT inclusions have been detected in cortical and striatal astrocytes (Shin et al. 2005). Although grade 0 showed no astrogliosis, it increased twofold in grade 2 and above tissues (Faideau et al. 2010) as shown by the GFAP immunostaining. The presence of mHTT aggregates in astrocytes caused decreased expression of GLT1/EAAT2 glutamate transporters (Arzberger et al. 1997). The GLT1 transporters are necessary for glutamate uptake, and loss of these results in neuronal excitotoxicity (Shin et al. 2005) (Khakh et al. 2017) (Faideau et al. 2010). A constant finding in the major studies reports loss of GLT1 in HD (Shin et al. 2005; Arzberger et al. 1997). Hence

loss of GLT1 causes astrocytic dysfunction due to the presence of mHTT aggregates. Many studies involving HD mice model have proven that mHTT expressing astrocytes have a key role in HD pathology (Bradford et al. 2010; Khakh et al. 2017). Expression of mHTT in astrocytes cocultured with striatal neurons resulted in the death of the latter cells (Cho et al. 2019) (Shin et al. 2005). In a study by Miller and colleagues, ceftriaxone (known to increase expression of GLT1) when injected to R6/2 model of HD mice showed upregulated GLT1 expression and hence increase in glutamate uptake with attenuation of HD phenotype (Miller et al. 2008). Along with GLT1, another inward rectifying K^+ (Kir4.1) channel was reported to decrease in HD pathology (Tong et al. 2014). Kir4.1 expressed in astrocytes and are involved in K^+ homeostasis (Nwaobi et al. 2016). Tong and colleagues (Tong et al. 2014) showed through a series of experiments that striatal astrocytes of WT and R6/2 diseased animals displayed electrophysiological differences that can be attributed to Kir4.1 channel downregulation. When AAV2/5 mediated Kir4.1 was targeted to astrocytes, rescue in motor deficits was observed along with increased survival rate.

BDNF is an important neurotrophic factor which is important for neuronal survival and function. Normal astrocytes secrete BDNF, but due to the presence of mHTT in astrocytes, this function is also collapsed in HD (Hong et al. 2016). Haim and colleagues have reported the activation of JAK/STAT3 promotes astrocytic reactivity in several neurodegenerative disease models including HD. The JAK/STAT3 pathway contributes to the cytokine signaling in cells by regulating the expression of genes involved in cell growth, proliferation, differentiation, and inflammation. JAK/STAT3 pathway also regulates astrogliogenesis during brain development (He et al. 2005), by promoting the expression of mature astrocyte genes such as GFAP and S100β (Kanski et al. 2014). To highlight the role of glia in HD, recent studies showed using stem cell approaches in HD model mice that mHTT glia can induce disease phenotype to normal WT mice (Benraiss et al. 2016). They created a mice model where immunodeficient mice were engrafted with mHTT expressing human glial progenitor cells. They found that normal glia when neonatally engrafted into the striata of R6/2 mice, rescue of the disease phenotypes was observed along with delayed motor deficits in comparison to ungrafted mice.

Astrocytic dysfunction can be thought as a key component of HD pathophysiology. Due to the loss of normal astrocytic function and aggregation of mHTT in the cells, disease condition worsens in a progressive manner. Astrocytic inflammatory response also hampers the cerebrovascular function by lowering the survival of the pericytes (Hsiao et al. 2015). The studies mentioned in this chapter suggest a synergistic role of mHTT and astrocytic dysfunction in HD pathogenesis.

4 Therapeutics Targeting Neuroinflammatory Components

Studies involving pharmacological intervention in neuroinflammatory pathway have risen in the recent past. Parawexin, a molecule from spider venom, has been used to increase glutamate uptake by EAAT2 transporter (Sofroniew 2009). β-Lactam antibiotics like ceftriaxone has been proven to reduce excitotoxicity in stroke and

ALS models. Minocycline, which is a well-known antibiotic to act on pro-inflammatory pathway, had shown promising results in the initial phases of clinical trial in HD patients. This compound lowered the motor and psychiatric symptoms (Bonelli et al. 2004), but the drug failed to reach the desired improvement and hence was clinically disapproved. Another compound, laquinimod, was proven to reduce the production of pro-inflammatory cytokines and resulted in improved motor function in YAC128 mice (Garcia-Miralles et al. 2016). HTT transcription and its mRNA translation are an area that has a great therapeutic potential apart from targeting neuroinflammatory components. Certain antisense oligonucleotides, RNAi targeting techniques, and CRISPR-Cas9 methods are under investigation. An intrathecally delivered antisense oligonucleotide, RG6042, is in phase I human clinical trial (Wild and Tabrizi 2017) (Tabrizi et al. 2018). A better understanding of the highly heterogeneous nature of microglia and astrocytes is required for successful clinical trial of molecules targeting neuroinflammatory components in HD and other diseases as well.

5 Conclusions

Microglia and astrocytes prove to be the defense system of the brain against any insult like mHTT (described in Fig. 24.1). But a chronic activation of these cell types leads to deterioration and collapse of the normal functioning of the brain. Neurons have always been at the receiving end of the ill effects of this detrimental process. Most of the studies in neuroinflammation emphasize on the dynamic interaction

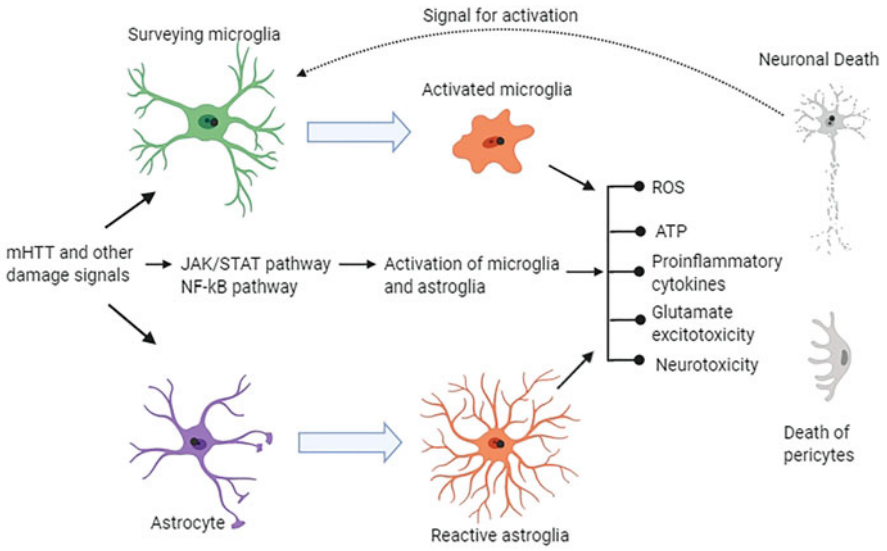

Fig. 24.1 Possible involvement of both microglia and astrocytes in inducing inflammatory process in HD

between the glia and neurons in normal as well as diseased state. Individually, the role of different cells in neuroinflammation has been well studied. It is important to note that none of the individual components of neuroinflammatory process exist in isolation; therefore, more experiments should be designed to target the interaction between cells. Some studies also hinted neuronal release of various inflammatory mediators in HD cellular model and hence could initiate inflammatory processes. Better understanding of the role of neurons in initiating inflammatory response could also be helpful in dissecting the role of neuroinflammation in HD pathogenesis.

Funding Information This work was supported by the core grant of NBRC provided by the Department of Biotechnology, Govt. of India, as well as from the ISIRD, SRIC of IIT Kharagpur.

References

Arzberger T, Krampfl K, Leimgruber S, Weindl A (1997) Changes of NMDA receptor subunit (NR1, NR2B) and glutamate transporter (GLT1) mRNA expression in Huntington's disease—an in situ hybridization study. J Neuropathol Exp Neurol 56(4):440–454

Belanger M, Magistretti PJ (2009) The role of astroglia in neuroprotection. Dialogues Clin Neurosci 11(3):281–295

Benarroch EE (2013) Microglia: multiple roles in surveillance, circuit shaping, and response to injury. Neurology 81(12):1079–1088

Benraiss A, Wang S, Herrlinger S, Li X, Chandler-Militello D, Mauceri J, Burm HB, Toner M, Osipovitch M, Jim XQ, Ding F, Wang F, Kang N, Kang J, Curtin PC, Brunner D, Windrem MS, Munoz-Sanjuan I, Nedergaard M, Goldman SA (2016) Human glia can both induce and rescue aspects of disease phenotype in Huntington disease. Nat Commun 7:11,758

Biber K, Neumann H, Inoue K, Boddeke HW (2007) Neuronal 'On' and 'Off' signals control microglia. Trends Neurosci 30(11):596–602

Blackburn D, Sargsyan S, Monk PN, Shaw PJ (2009) Astrocyte function and role in motor neuron disease: a future therapeutic target? Glia 57(12):1251–1264

Bonelli RM, Hodl AK, Hofmann P, Kapfhammer HP (2004) Neuroprotection in Huntington's disease: a 2-year study on minocycline. Int Clin Psychopharmacol 19(6):337–342

Boscia F, Esposito CL, Di Crisci A, de Franciscis V, Annunziato L, Cerchia L (2009) GDNF selectively induces microglial activation and neuronal survival in CA1/CA3 hippocampal regions exposed to NMDA insult through Ret/ERK signalling. PLoS One 4(8):e6486

Bradford J, Shin JY, Roberts M, Wang CE, Sheng G, Li S, Li XJ (2010) Mutant huntingtin in glial cells exacerbates neurological symptoms of Huntington disease mice. J Biol Chem 285(14): 10,653–10,661

Calabresi P, Centonze D, Pisani A, Sancesario G, Gubellini P, Marfia GA, Bernardi G (1998) Striatal spiny neurons and cholinergic interneurons express differential ionotropic glutamatergic responses and vulnerability: implications for ischemia and Huntington's disease. Ann Neurol 43(5):586–597

Chen JY, Parekh M, Seliman H, Bakshinskaya D, Dai W, Kwan K, Chen KY, Liu AYC (2018) Heat shock promotes inclusion body formation of mutant huntingtin (mHtt) and alleviates mHtt-induced transcription factor dysfunction. J Biol Chem 293(40):15,581–15,593

Cho IK, Yang B, Forest C, Qian L, Chan AWS (2019) Amelioration of Huntington's disease phenotype in astrocytes derived from iPSC-derived neural progenitor cells of Huntington's disease monkeys. PLoS One 14(3):e0214156

Chung W-S, Allen NJ, Eroglu C (2015) Astrocytes control synapse formation, function, and elimination. Cold Spring Harb Perspect Biol 7(9):a020370

Crapser JD, Ochaba J, Soni N, Reidling JC, Thompson LM, Green KN (2020) Microglial depletion prevents extracellular matrix changes and striatal volume reduction in a model of Huntington's disease. Brain 143(1):266–288

Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auregan G, Dufour N, Guillermier M, Brouillet E, Hantraye P, Deglon N, Ferrante RJ, Bonvento G (2010) In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. Hum Mol Genet 19(15):3053–3067

Garcia-Miralles M, Hong X, Tan LJ, Caron NS, Huang Y, To XV, Lin RY, Franciosi S, Papapetropoulos S, Hayardeny L, Hayden MR, Chuang KH, Pouladi MA (2016) Laquinimod rescues striatal, cortical and white matter pathology and results in modest behavioural improvements in the YAC128 model of Huntington disease. Sci Rep 6:31,652

Giralt A, Saavedra A, Carreton O, Xifro X, Alberch J, Perez-Navarro E (2011) Increased PKA signaling disrupts recognition memory and spatial memory: role in Huntington's disease. Hum Mol Genet 20(21):4232–4247

Guidetti P, Luthi-Carter RE, Augood SJ, Schwarcz R (2004) Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. Neurobiol Dis 17(3):455–461

Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE, Watkins PC, Ottina K, Wallace MR, Sakaguchi AY, Young AB, Shoulson I, Bonilla E, Martin JB (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306(5940): 234–238

van Hagen M, Piebes DGE, de Leeuw WC, Vuist IM, van Roon-Mom WMC, Moerland PD, Verschure PJ (2017) The dynamics of early-state transcriptional changes and aggregate formation in a Huntington's disease cell model. BMC Genomics 18(1):373

Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG (2007) Synaptic islands defined by the territory of a single astrocyte. J Neurosci 27(24):6473–6477

Hanisch UK (2013) Functional diversity of microglia—how heterogeneous are they to begin with? Front Cell Neurosci 7:65

Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394

He F, Ge W, Martinowich K, Becker-Catania S, Coskun V, Zhu W, Wu H, Castro D, Guillemot F, Fan G, de Vellis J, Sun YE (2005) A positive autoregulatory loop of Jak-STAT signaling controls the onset of astrogliogenesis. Nat Neurosci 8(5):616–625

Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, Elliston LA, Hartog C, Goldstein DR, Thu D, Hollingsworth ZR, Collin F, Synek B, Holmans PA, Young AB, Wexler NS, Delorenzi M, Kooperberg C, Augood SJ, Faull RL, Olson JM, Jones L, Luthi-Carter R (2006) Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 15(6):965–977

Hong Y, Zhao T, Li XJ, Li S (2016) Mutant huntingtin impairs BDNF release from astrocytes by disrupting conversion of Rab3a-GTP into Rab3a-GDP. J Neurosci 36(34):8790–8801

Hsiao HY, Chen YC, Huang CH, Chen CC, Hsu YH, Chen HM, Chiu FL, Kuo HC, Chang C, Chern Y (2015) Aberrant astrocytes impair vascular reactivity in Huntington disease. Ann Neurol 78(2):178–192

Imarisio S, Carmichael J, Korolchuk V, Chen CW, Saiki S, Rose C, Krishna G, Davies JE, Ttofi E, Underwood BR, Rubinsztein DC (2008) Huntington's disease: from pathology and genetics to potential therapies. Biochem J 412(2):191–209

Jansen AH, van Hal M, Op den Kelder IC, Meier RT, de Ruiter AA, Schut MH, Smith DL, Grit C, Brouwer N, Kamphuis W, Boddeke HW, den Dunnen WF, van Roon WM, Bates GP, Hol EM, Reits EA (2017) Frequency of nuclear mutant huntingtin inclusion formation in neurons and glia is cell-type-specific. Glia 65(1):50–61

Joshi AU, Minhas PS, Liddelow SA, Haileselassie B, Andreasson KI, Dorn GW 2nd, Mochly-Rosen D (2019) Fragmented mitochondria released from microglia trigger A1 astrocytic response and propagate inflammatory neurodegeneration. Nat Neurosci 22(10):1635–1648

Kanski R, van Strien ME, van Tijn P, Hol EM (2014) A star is born: new insights into the mechanism of astrogenesis. Cell Mol Life Sci 71(3):433–447
Kettenmann H, Kirchhoff F, Verkhratsky A (2013) Microglia: new roles for the synaptic stripper. Neuron 77(1):10–18
Khakh BS, Beaumont V, Cachope R, Munoz-Sanjuan I, Goldman SA, Grantyn R (2017) Unravelling and exploiting astrocyte dysfunction in Huntington's disease. Trends Neurosci 40(7):422–437
Khoshnan A, Ko J, Watkin EE, Paige LA, Reinhart PH, Patterson PH (2004) Activation of the IkappaB kinase complex and nuclear factor-kappaB contributes to mutant huntingtin neurotoxicity. J Neurosci 24(37):7999–8008
Kraft AD, Kaltenbach LS, Lo DC, Harry GJ (2012) Activated microglia proliferate at neurites of mutant huntingtin-expressing neurons. Neurobiol Aging 33(3):621.e617–621.e633
Li JY, Plomann M, Brundin P (2003) Huntington's disease: a synaptopathy? Trends Mol Med 9(10):414–420
Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Munch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541(7638):481–487
MacVicar BA, Newman EA (2015) Astrocyte regulation of blood flow in the brain. Cold Spring Harb Perspect Biol 7(5):1–15
Mandrekar-Colucci S, Karlo JC, Landreth GE (2012) Mechanisms underlying the rapid peroxisome proliferator-activated receptor-gamma-mediated amyloid clearance and reversal of cognitive deficits in a murine model of Alzheimer's disease. J Neurosci 32(30):10,117–10,128
Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87(3): 493–506
Miller BR, Dorner JL, Shou M, Sari Y, Barton SJ, Sengelaub DR, Kennedy RT, Rebec GV (2008) Up-regulation of GLT1 expression increases glutamate uptake and attenuates the Huntington's disease phenotype in the R6/2 mouse. Neuroscience 153(1):329–337
Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8(12):958–969
Nedergaard M, Ransom B, Goldman SA (2003) New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci 26(10):523–530
Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318
Nwaobi SE, Cuddapah VA, Patterson KC, Randolph AC, Olsen ML (2016) The role of glial-specific Kir4.1 in normal and pathological states of the CNS. Acta Neuropathol 132(1):1–21
Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M (2009) Uniquely hominid features of adult human astrocytes. J Neurosci 29(10):3276–3287
Olzscha H, Schermann SM, Woerner AC, Pinkert S, Hecht MH, Tartaglia GG, Vendruscolo M, Hayer-Hartl M, Hartl FU, Vabulas RM (2011) Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. Cell 144(1):67–78
Palpagama TH, Waldvogel HJ, Faull RLM, Kwakowsky A (2019) The role of microglia and astrocytes in Huntington's disease. Front Mol Neurosci 12(October):258
Pekny M, Pekna M, Messing A, Steinhauser C, Lee JM, Parpura V, Hol EM, Sofroniew MV, Verkhratsky A (2016) Astrocytes: a central element in neurological diseases. Acta Neuropathol 131(3):323–345
Persson M, Ronnback L (2012) Microglial self-defence mediated through GLT-1 and glutathione. Amino Acids 42(1):207–219
Phatnani H, Maniatis T (2015) Astrocytes in neurodegenerative disease. Cold Spring Harb Perspect Biol 7(6):1–18

Ransohoff RM, Cardona AE (2010) The myeloid cells of the central nervous system parenchyma. Nature 468(7321):253–262

Reiner A, Albin RL, Anderson KD, D'Amato CJ, Penney JB, Young AB (1988) Differential loss of striatal projection neurons in Huntington disease. Proc Natl Acad Sci U S A 85(15):5733–5737

Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N, Kuncl RW (1994) Localization of neuronal and glial glutamate transporters. Neuron 13(3):713–725

Rouach N, Koulakoff A, Giaume C (2004) Neurons set the tone of gap junctional communication in astrocytic networks. Neurochem Int 45(2–3):265–272

Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, Bhide PG, Vonsattel JP, DiFiglia M (2001) Early and progressive accumulation of reactive microglia in the Huntington disease brain. J Neuropathol Exp Neurol 60(2):161–172

Shin JY, Fang ZH, Yu ZX, Wang CE, Li SH, Li XJ (2005) Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. J Cell Biol 171(6):1001–1012

Simmons DA, Casale M, Alcon B, Pham NHA, Narayan N, Lynch G (2007) Ferritin accumulation in dystrophic microglia is an early event in the development of Huntington's disease. Glia 55(10):1074–1084

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32(12):638–647

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119(1): 7–35

Tabrizi SJ, Leavitt B, Landwehrmeyer B, Wild E, Saft C, Barker R, Craufurd D, Rickards H, Rosser A, Priller J, Kordasiewicz H, Czech C, Swayze E, Norris DA, Baumann T, Gerlach I, Schobel S, Smith A, Lane R, Bennett CF (2018) J01 effects of IONIS-HTTRX (RG6042) in patients with early huntington's disease, results of the first htt-lowering drug trial. J Neurol Neurosurg Psychiatry 89(Suppl 1):A97–A98

Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, Piccini P (2007) Microglial activation in presymptomatic Huntington's disease gene carriers. Brain 130(Pt 7):1759–1766

Tong X, Ao Y, Faas GC, Nwaobi SE, Xu J, Haustein MD, Anderson MA, Mody I, Olsen ML, Sofroniew MV, Khakh BS (2014) Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. Nat Neurosci 17(5):694–703

Trager U, Andre R, Lahiri N, Magnusson-Lind A, Weiss A, Grueninger S, McKinnon C, Sirinathsinghji E, Kahlon S, Pfister EL, Moser R, Hummerich H, Antoniou M, Bates GP, Luthi-Carter R, Lowdell MW, Bjorkqvist M, Ostroff GR, Aronin N, Tabrizi SJ (2014) HTT-lowering reverses Huntington's disease immune dysfunction caused by NFkappaB pathway dysregulation. Brain 137(Pt 3):819–833

Verkhratsky A, Nedergaard M (2018) Physiology of astroglia. Physiol Rev 98(1):239–389

Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57(5):369–384

Voskuhl RR, Peterson RS, Song B, Ao Y, Morales LB, Tiwari-Woodruff S, Sofroniew MV (2009) Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. J Neurosci 29(37):11,511–11,522

Wild EJ, Tabrizi SJ (2017) Therapies targeting DNA and RNA in Huntington's disease. Lancet Neurol 16(10):837–847

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32(18):6391–6410

Endogenous Mediators of Neuroinflammation: The Endocannabinoid System at the Retina

Durairaj Duraikkannu and Nivedita Chatterjee

Abstract

The retina is protected from the immune cells of the blood by the blood-retina barrier. In the retina, the immune functions are mediated by Muller glia, pericytes, retinal microglia, and astrocytes. Stringent regulation of signaling pathways and cellular interactions ascertain that both innate and adaptive immune responses do not overwhelm and cause tissue damage. The endogenous cannabinoid system and the lipid derivatives which make the most important ligands participate in multiple physiological processes that affect neuronal, immune, metabolic, and gastrointestinal regulatory mechanisms. Endocannabinoids (ECs) act as potent anti-inflammatory agent on immune cells of the blood and central nervous system (CNS). Endogenous cannabinoid levels change in several ocular diseases like glaucoma and diabetic retinopathy. In this review article, we describe some of the latest advances in our knowledge of the endocannabinoid system in the eye. We also describe from our investigations at the eye the importance of endocannabinoids and the endocannabinoid system genes, in modulating innate immune response of retinal Muller glia to create a pro-survival milieu.

Keywords

Retina · Endocannabinoid · Inflammation

D. Duraikkannu · N. Chatterjee (✉)
Department of L&T Ocular Pathology, Vision Research Foundation, Chennai, Tamil Nadu, India
e-mail: drnc@snmail.org

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_25

1 Introduction

"Endogenous cannabinoids" are part of an endocannabinoid system that encompasses endogenous ligands or endocannabinoids (eCB), the receptors to which they bind and the mediators responsible for their synthesis, metabolism, and catabolism. The endocannabinoid system consists of receptors for uptake and enzymes such as phospholipase-D (PLD) for generation and enzymes that hydrolyze the lipids, like fatty acid amide hydrolase (FAAH). Endocannabinoids are derived from integral components of the phospholipid bilayers of cellular membranes and are hydrophobic lipid molecules. The eCB family contains several structurally related lipids (Devane et al. 1992) which are synthesized and released by a wide variety of cells in the body. Acting in an autocrine and/or paracrine manner, these molecules locally influence many cellular functions (Scotter et al. 2010). Cannabinoid ligands are divided into four groups based on their chemical structure: classical, nonclassical, amino-alkylindoles, and eicosanoids. Amino acid derivatives of acyl fatty acids, like N-palmitoylethanolamine, 2-oleoylglycerol, N-arachidonoylglycine, as well as ethanolamine and glycerol, are also thought to act through the endocannabinoid system. N-arachidonoylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) are both eicosanoids. Cannabinoids mainly act through two distinct G-protein-coupled receptors, CB1 and CB2. Chemically, anandamide is the amide constituent of arachidonic acid and ethanolamine. Several pathways produce AEA, including the eicosanoid biosynthetic enzymes, cyclooxygenases. However, N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), which catalyzes the breakage of N-acylethanolamine from N-arachidonoyl-phosphatidylethanolamine, is critical for AEA biosynthesis (Di Marzo et al. 1994). AEA binds to receptors in the brain and at peripheral sites across the body. The other major endocannabinoid is 2-arachidonoylglycerol (Mechoulam et al. 1995). 2-AG has been found to be the most bioactive and abundant endocannabinoid in the brain and eye, with concentrations reported as considerably higher than that of anandamide (Bisogno et al. 1999a, b). 2-AG is synthesized by the hydrolysis of 1,2-diacylglycerol (DAG) through DAG lipase (DAGL)α and β or 2-arachidonoyl-lysophospholipid by lyso-phospholipase (PLC). 2-AG also is apparently transported across the plasma membrane before it is degraded by FAAH or by the serine hydrolase, monoacylglycerol lipase (MAGL). FAAH is also the main degrading enzyme of AEA. Very like neurotransmitters, the generation, uptake, and degradation of endocannabinoids are rapid.

While the dominant receptors in most tissues remains CB1 and CB2, double-knockout mice have revealed non-CB1/CB2 receptor-mediated cannabinoid effects (discussed in Scotter et al. 2010). Potential candidate receptors include the orphan G protein-coupled receptor GPR55, GPR18, transient receptor potential vanilloid type-1 (TRPV1) channel, and the nuclear receptor superfamily of peroxisome proliferator-activated receptors (PPARs) (Rockwell and Kaminski 2004).

2 Endocannabinoids as Immunomodulators in the CNS

In the brain, the main pharmacological function of the endocannabinoid system is thought to be neuromodulation (Bisogno et al. 1999a, b; Di Marzo et al. 1998), controlling motor functions, cognition, emotional responses, homeostasis, and motivation. They play a postsynaptic regulatory role by modulating the release of several neurotransmitters such as gamma-aminobutyric acid (GABA), glutamate, and dopamine. Stress induced increase in FAAH can reduce AEA and prevent the increase in synaptic activation and delayed synaptic strengthening in the amygdala (Yasmin et al. 2020). Similarly, the endocannabinoid system is an important modulator of the autonomic nervous system (ANS). ECS has also been shown to alleviate pain by modulating sensory nociception (Kaur et al. 2020). Through anecdotal evidence, however, it soon was evident that the cannabinoids play an equally important immune regulatory function (Yazulla 2008). Immunomodulatory roles of the endocannabinoid system were initially studied in the peripheral immune system (Di Marzo et al. 1998). The immunosuppressive properties of endocannabinoids are exerted through induction of apoptosis, regulation in production of pro-inflammatory factors, and inhibition of cell proliferation (Cabral and Griffin-Thomas 2008). Potent anti-inflammatory effects of endocannabinoids are now known to occur in both immune cells of the blood and brain (Maccarrone et al. 2015). Neuroinflammatory processes have both protective and detrimental effects in the brain. Microglia, as the primary immune effector cells in the central nervous system, has been extensively studied (reviewed in Tanaka et al. 2020) for their roles in a variety of neuroinflammatory conditions. On stimulation of the endocannabinoid system, they proliferate and migrate to sites of infection, inflammation, or tissue injury (Sawada et al. 1990; Walter et al. 2003) and can change their activation state.

Cannabinoid agonists are being used for treatment of neuropathic pain, cancer pain (Chaperon and Thiébot 1999; Bradshaw and Walker 2005; Fowler et al. 2005; Lambert and Fowler 2005), and spasticity associated with MS (da Rovare et al. 2017). Positive results of administration of cannabinoid agonists have been observed in Parkinson's disease patients (Papa 2008), with further trials revealing beneficial effects of cannabinoids in Alzheimer's disease patients (Dhawan et al. 2008). Chronic exposure to cannabinoid receptor CB1 agonists has been reported to induce downregulation of the CB1 receptor in the brain and behavioral tolerance. Endocannabinoids are also emerging as instructive cues in the developing central nervous system, and based on the expression of their receptors and metabolizing enzymes, oligodendrocytes are shown to use these molecules (Ilyasov et al. 2018).

3 Endocannabinoids in Ocular Diseases

In pathological conditions such as diabetic retinopathy (DR) and senescence changes such as age-related macular degeneration (AMD), oxidative stress is a key component (Behl and Kotwani 2015; Behl et al. 2016, discussed by Forest et al. 2015). In a

rat model of DR, cannabidiol addition significantly suppressed oxidative stress and neurotoxin production, thus improving retinal cell health. AEA levels increase in AMD (Matias et al. 2006). In an in vitro study model of AMD, antagonists to CB had a protective effect on retinal pigment epithelium cells from oxidative damage (Wei et al. 2013). Creation of a protective milieu by cannabinoids was also seen in an animal model for autosomal dominant retinitis pigmentosa (RP). In this model, photoreceptor degeneration decreased along with improved synaptic connectivity and functional activity at the retina (Lax et al. 2014) on cannabinoid administration. Much work has been done in glaucoma where control of glutamate induced excitotoxicity and decreasing intraocular pressure (IOP) through cannabinoid treatments suggest control of the disease at multiple levels (Chen et al. 2005). In eyes from patients with glaucoma, 2-AG and palmitoylethanolamide (PEA) levels which were detected in the ciliary body showed a sharp fall. Excessive extracellular glutamate release including enhanced production by Muller cells had been identified as one of the pathophysiological mechanisms in glaucoma (Ishikawa et al. 2015). Administration of THC and cannabidiol limited generation of peroxynitrite production in a rat model of excitotoxicity consisting in intravitreal injection of N-methyl-D-aspartate (NMDA) (El-Remessy et al. 2003). Manipulation of CB receptors and degradation enzymes has shown a positive outcome in glaucoma. These data argue for the use of cannabinoids in retinal dysfunction as an adjunct neuro-therapeutic agent to generate a pro-survival environment and limit neurodegeneration.

4 Endocannabinoid System in the Retina

The presence of a functional endocannabinoid system in eye tissue supports multiple roles for endocannabinoids in ocular physiology. The regulatory role of the cannabinoid system in the retinal neurotransmission occurs at several layers: photoreceptor, bipolar, and ganglion cells. Receptors and enzymes for endocannabinoids have been identified in retinas of rat, cat, and monkey (reviewed in Schwitzer et al. 2016). 2-AG, AEA, and PEA have all been identified in ocular tissues of various species. 2-AG remains the most abundant endocannabinoid in eye tissue (Straiker et al. 1999; Stamer et al. 2001). Evidence from many studies confirmed that cannabinoid agonists modulate retinal neuronal transmission. In a dose-dependent reversible manner, these agonists control calcium, potassium, and chloride currents. This implies a neuromodulatory role of the cannabinoid system in moderating the vertical transmission of the retinal information and consequently altering visual perception (discussed in Schwitzer et al. 2016). One study propounded that the endocannabinoid system has developmental stage-dependent role in the maturation of synaptic retinal circuits (Middleton and Protti 2011). The functions of endocannabinoids in the eye include regulation of photoreception, neurotransmission in the optic nerve (Schlicker et al. 1996; Fan and Yazulla 2003; Straiker and Sullivan 2003), and protective action as observed in an experimental allergic uveitis model (Pryce et al. 2003). AEA and PEA through the TRPV1 receptors are now thought to play a role in retinal microcirculation (Yazulla and Studholme 2004).

More studies are required to detect the roles of the several putative receptors GPR55, GPR18, TRPV1, and PPAR and purinergic receptors P2X7 (discussed in Di Marzo 2018) in the eye, as these proteins are already part of various signaling components.

In the eye, changes in endocannabinoid levels and the genes have been particularly well studied for glaucoma and diabetic retinopathy. Addition of cannabinoid agonists is known to lower IOP in rabbits, nonhuman primates, and glaucomatous humans (Järvinen et al. 2002). Endocannabinoids can provide tonic regulation of IOP by the association of ocular hypotension. Inhibition of FAAH and consequent increases in endocannabinoid levels provide a route to regulate IOP (Laine et al. 2001; Laine et al. 2002). In glaucoma, toxic effects accrue on increased release of the neurotransmitter glutamate. Growing evidence support a role of ECS in aqueous humor dynamics and that drugs targeting both CB1 and noncanonical receptors may prove useful as ocular hypotensives. Endogenous and synthetic cannabinoids have been shown to provide neuroprotection to retinal neurons in acute animal models of retinopathy.

5 Modulation of Muller Glia Immune Response by Endocannabinoids and Its Implications in Glial Physiological Functions

The retina employs its resident glial cells (microglia, Muller glia, and astrocytes) to control immunity. This is a complex physiological function where stringent control is essential in order to limit damage to the tissue through overwhelming inflammation. Muller glia, the predominant retinal glia, is actively involved in many inflammatory conditions of the retina. Accumulating evidence from our and several other groups now show that activation of Muller glia has an innate immune component (Shamsuddin and Kumar 2011; Krishnan and Chatterjee 2012). Muller glia respond to inflammation by secreting a variety of neurotrophic factors, antioxidants, and inflammatory mediators. They play a prominent role in creating a neuroprotective milieu. Activated Muller glia exposed to stressors like LPS and HIV1 Transactivator protein (Tat) show elevated production of pro-inflammatory factors, potential neurotoxins that can cause retinal degeneration. Our group has looked at Muller glial innate immune response over a variety of stressors. For example, HIV1 pathology is correlated with cytokine/chemokine dysregulation, including in the eye (Hofman and Hinton 1992). We showed that activated retinal cell R28 produced copious amounts of chemokines and cytokines (Chatterjee et al. 2011) which had the ability to chemo-attract macrophages, a potential pathway of disease progression.

Falling levels of AEA and 2-AG are associated with neuronal death at the brain. On the basis of endocannabinoid functions on other glial cells in the CNS (Witting et al. 2006; Eljaschewitsch et al. 2006), we found out how in retinal Muller cells, endocannabinoids, AEA, and 2-AG regulated to decrease pro-inflammatory cytokines while selectively upregulating production of anti-inflammatory factors (Krishnan and Chatterjee 2012). We set out to dissect how the retina can provide a pro-survival milieu and investigated in detail the contributory role of Muller glia. We

identified the signaling pathways involved in this switch toward an anti-inflammatory milieu and demonstrated the critical roles of mitogen-activated protein kinases (MAPKs) and the canonical NF-κB signaling in this process. CB receptors as GPCRs are capable of recruiting a complex set of intracellular protein kinases involved in gene expression. Pro-inflammatory responses of Muller glia to lipopolysaccharide (LPS) is mediated by Toll-like receptors (TLR), several isoforms of which (discussed in Downer et al. 2011) Muller cells possess (Kumar et al. 2013). Endocannabinoids can ablate this activation. Endocannabinoid action in alleviating this activation by LPS is through CB1 and CB2 receptors. Addition of AEA and 2-AG modulates phosphorylation of ERK, JNK, and p38, the most important MAPK enzymes. The phosphorylation and duration of phosphorylation of these enzymes show a shift in activated Muller glia exposed to AEA and 2-AG. Furthermore, inhibitors to ERK, JNK, and p38 abrogate the LPS and endocannabinoid induced changes in cytokine levels (Krishnan and Chatterjee 2012; Krishnan and Chatterjee 2014). We also observed that the negative regulator of MAPK phosphorylation, MAPK phosphatases (MKP), shows increased levels on coincubation with LPS and AEA. MKP-1 plays a critical role in their AEA-mediated production of anti-inflammatory cytokines at the late phase of inflammation by affecting JNK and p38 expressions. The endocannabinoids also affect the NF-κB signalosome. Our data showed that inhibitors to MAPK components lead to reversal in IκB phosphorylation even with parallel stimulation of LPS and either of the endocannabinoids. We suggest that the reduced translocation into the nucleus is possibly responsible for the enhanced production of IL-10 (an anti-inflammatory cytokine) while suppressing the generation of the pro-inflammatory cytokines such as TNF-α, IL-6, and IL-12, which are influenced by NF-κB (Hirotani et al. 2005). Additionally, inhibitory protein components of the NF-κB super complex such as Interleukin 1 Receptor Associated Kinase 1 Binding Protein 1 (IRAK1BP1) are regulated by AEA and 2-AG. Increase in IRAK1BP1 on exposure to AEA or 2-AG during inflammation possibly shifts the balance of NF-κB subunits available for promoter binding to transcriptionally responsive genes. It is significant that the retina as an immune-privileged organ has elevated levels of IRAK1BP1 inherently and exogenous addition of eCB increases it further under inflammatory conditions.

Since endocannabinoids are known to act as neuroprotectants and alter blood-brain barrier (BBB) permeability in various neurological disorders, several laboratories have investigated endocannabinoids on BBB permeability in normal and ischemic conditions (Hind et al. 2015; Piro et al. 2018). ECS components including 2-AG, oleoylethanolamine (OEA), and PEA (Hillard 2008; Naccarato et al. 2010) increase during stroke. In an in vitro model investigating BBB, the group of Samad (Piro et al. 2018) showed an effect on MAGL inhibition in preserving BBB function acting through reduction of arachidonic acid generation. The investigators suggested MAGL inhibitors as potential therapeutics to prevent BBB breakdown due to inflammation. In an in vitro BBB model with human brain microvascular endothelial cell and astrocyte cocultures, where ischemia was modeled by oxygen-glucose deprivation, exogenous AEA and OEA improved barrier properties (Hind et al. 2015).

Significant amount of work exists in the field of blood-brain barrier, demonstrating the role of nitric oxide (NO) in modulation of barrier properties (Thiel and Audus 2001; Zhang et al. 2014). At the inner blood-retina barrier (iBRB), Muller cell end feet along with pericytes, astrocytes, and endothelial cells form a boundary with the local vasculature. In Muller glia, we explored how excess NO production by these cells influences junctional proteins and therefore permeability. Tight junction complexes at the iBRB are mega-protein platforms, among them, Cx43 and zonula occludens-1 (Laing et al. 2005). Activation by LPS changes both these proteins relative to untreated control cells. Fractionation of cellular organelles from enriched cultures of Muller cells showed plasma membrane with reduced ZO-1 expression. We further showed that nuclear ZO-1 tends to rise on addition of LPS, whereas addition of AEA reduces sequestration in the nucleus. Thus, we showed that readily available ZO-1 falls during inflammation likely affecting barrier behavior (Krishnan and Chatterjee 2015a). Though on a simplified model, this study implies an autocrine and paracrine mode of action by eCB for Muller cells on BRB function. Disruption of junction proteins can increase monocyte and leukocyte trafficking which in turn can exacerbate the inflammation (Krishnan and Chatterjee 2015b). Endogenous cannabinoids and cannabi-mimetics can therefore be considered for adjunct therapy in disorders of the eye with an immunopathology where the blood-retina barrier is affected.

6 Inflammation and Changes in Endocannabinoid System Genes in Muller Glia

The endocannabinoid system genes require stimulation for the various physiological functions. Endocannabinoids elicit localized effects that are relatively short-lived. The enzymes involved in production and degradation of the endocannabinoids therefore function "on-demand." In animal models of excitotoxic lesions, there is intrinsic upregulation of 2-AG which is synergistically controlled by DAGLB and MAGL in both neurons and astrocytes, thus providing a protective system in the brain (Kallendrusch et al. 2012). In a spinal cord contusion model in rats (Garcia-Ovejero et al. 2009), lesions showed increase of PEA and AEA levels, upregulated CB2 receptor expression after lesion occurred, and in the same area elevated production in the synthesizing enzyme NAPEPD and PLD and downregulated levels of the degradative enzyme FAAH.

We looked at the endocannabinoid system genes: CB1, CB2, synthesis enzymes, DAGLB and NAPEPD, and catabolic enzyme, FAAH. Activated Muller glia do not show unequivocal dose-dependent changes in all genes (Fig. 25.1). CB1 and CB2 show the most prominent changes after induction by the stressor LPS showing a sustained increase as also DAGLB (Fig. 25.1c). In the activation model with LPS, a consistent increase in the expression of all the genes studied is observed (Fig. 25.2) at 2, 6, 8, and 24 h. LPS also increases the degradative enzyme FAAH only at 24 hours. Addition of AEA exogenously however leads to certain upregulation only in CB1 (Fig. 25.3a) and CB2 (Fig. 25.3b) and only a slight fall in FAAH

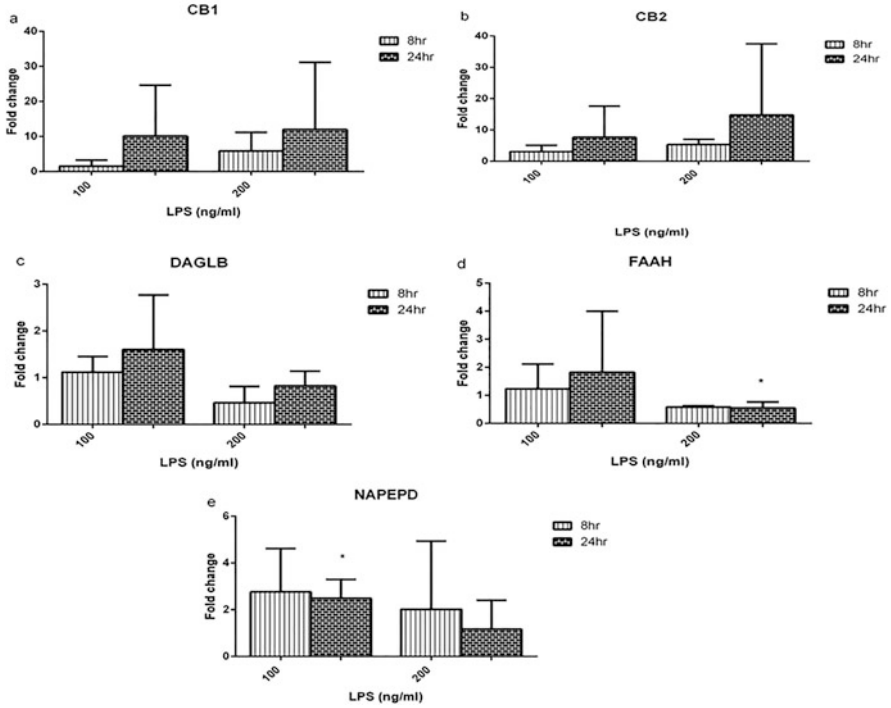

Fig. 25.1 Dose-dependent changes in endocannabinoid receptor genes on primary bovine Muller glia stimulated with lipopolysaccharide. Endocannabinoid genes with increasing concentration of LPS (100 and 200 ng/mL) at two time points (8 and 24 h). While CB1 (**a**) and CB2 (**b**) show increased expression at 200 ng/mL LPS, the other genes do not. There is a greater expression of CB1 and CB2 at 24 h. DAGLB (**c**), FAAH (**d**), and NAPEPD (**e**) show highest expression at 8 h and do not show dose-dependent changes. At 200 ng/mL on 8 h treatment $p < 0.01$

(Fig. 25.3d). The other enzymes are suppressed on additional stimulus from AEA. The CB1 and CB2 antagonists, AM251 and AM630 (Fig. 25.3), moreover only cause partial reversal of the effects on Muller glia of LPS and AEA coincubation. DAGLB is strongly suppressed by AEA (Fig. 25.3c). Reversal of AEA effect in activated Muller glia is most conspicuous on addition of AM630 (Fig. 25.3a, b, d) when compared to AM251. Both CB1 (Fig. 25.3a) and CB2 (Fig. 25.3b) levels which rise with AEA are suppressed on addition of AM630. FAAH instead rises on addition of AM630 (Fig. 25.3d). In effect, the rise in CB receptors and fall in FAAH may help generate a protective milieu.

The differential effects on the ECS genes, on addition of AEA in activated cells, are noteworthy since the ECS pathway can be manipulated accordingly with specific antagonists or agonists. While the challenge to design drugs to target a particular tissue and without psychoactive effects remain, identification and localization of the ECS components in the eye including non-GPCR CB receptors can alleviate overwhelming and detrimental inflammation.

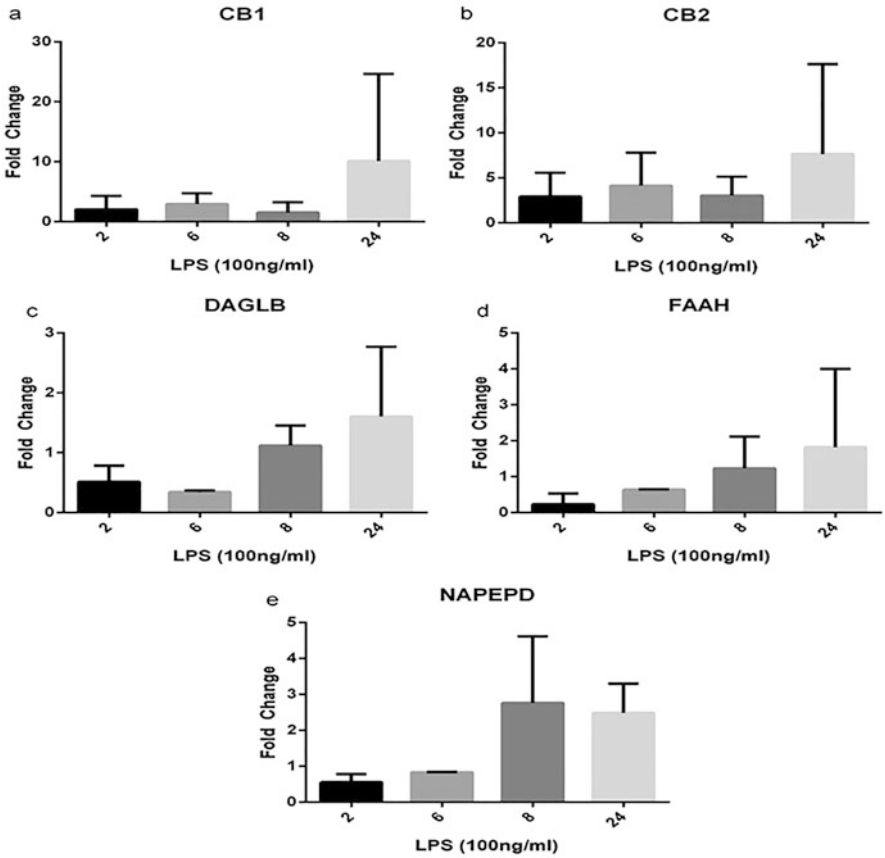

Fig. 25.2 Time kinetics of endocannabinoid system genes in stimulated Muller glia. CB1 (**a**), CB2 (**b**), DAGLB (**c**), FAAH (**d**), and NAPEPD (**e**) all show consistent rise in mRNA in stimulated retinal Muller glia. Treatment of cells was with 100 ng/mL LPS for designated time points

7 Conclusion

Endocannabinoids and their associated genes are found in all components of the eye and the neural retina. They can be detected in photoreceptor cells, bipolar cells, ganglion cells, amacrine, horizontal neurons, as well as Muller glia and retinal pigment epithelium cells. ECS is implicated in retinal neurotransmission, neuroplasticity, and neuroprotective physiological functions. Animal models of retinal disorders have emphasized that cannabinoid therapy and manipulating ECS components show a neuroprotective role. Numerous studies including from the authors' (Krishnan and Chatterjee 2012; Krishnan and Chatterjee 2014; Krishnan and Chatterjee 2015a; b) demonstrate the importance of glial contribution for retinal health and disease progression. This review detailed the relevance of the

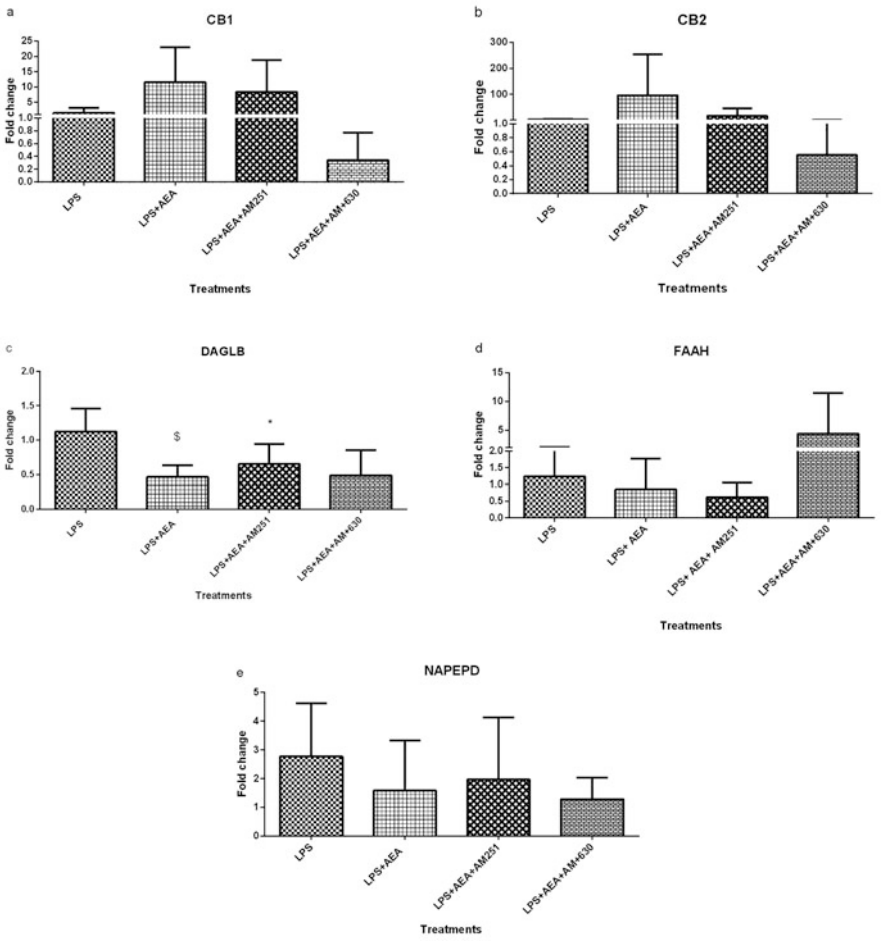

Fig. 25.3 Effect of exogenous AEA on endocannabinoid system genes in activated Muller glia. Addition of AEA on primary bovine Müller glia shows strong upregulation of CB1 (**a**) and CB2 (**b**) receptors in coincubated LPS + AEA cells. Addition of CB1 receptor antagonist AM251 shows only partial reversal of AEA effect. In coincubated LPS + AEA cells, AEA suppresses production of (**c**) DAGLB, $p < 0.002$, (**d**) FAAH, and (**e**) NAPEPD. In all instances, AM251 reverses nominally LPS + AEA effect on the genes. Significant reversal is observed only in FAAH, $p < 0.05$ (**c**). Addition of CB2 receptor antagonist AM630 has a more pronounced reversal effect (**a**, **b**, **d**, **e**) though statistically not significant

endocannabinoid system in Muller glia in maintaining a healthy retina, the changes during inflammation, and the beneficial role played by Muller glia in the presence of endocannabinoids and strongly suggests curative uses of endogenous cannabinoids or cannabi-mimetic compounds in the treatment and the prevention of retinal diseases.

Funding Information This study was partially supported by the Indian Council of Medical Research, grant number Immun.18/11/20/2008-ECD-I, and the Department of Biotechnology, grant number BT/PR8585/AGR/36/780/2013 to N.C. D.D was supported by the Council of Scientific and Industrial Research.

References

Behl T, Kotwani A (2015) Exploring the various aspects of the pathological role of vascular endothelial growth factor (VEGF) in diabetic retinopathy. Pharmacol Res 99:137–148

Behl T, Kaur I, Kotwani A (2016) Role of endocannabinoids in the progression of diabetic retinopathy. Diabetes Metab Res Rev 32(3):251–259

Bisogno T, Berrendero F, Ambrosino G, Cebeira M, Ramos JA, Fernandez-Ruiz JJ, Di Marzo V (1999a) Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. Biochem Biophys Res Commun 256(2):377–380

Bisogno T, Melck D, De Petrocellis L, Di Marzo V (1999b) Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin. J Neurochem 72(5):2113–2119

Bradshaw HB, Walker JM (2005) The expanding field of cannabimimetic and related lipid mediators. Br J Pharmacol 44(4):459–465

Cabral GA, Griffin-Thomas L (2008) Cannabinoids as therapeutic agents for ablating neuroinflammatory disease. Endocr Metab Immune Disord Drug Targets 8(3):159–172

Chaperon F, Thiébot MH (1999) Behavioral effects of cannabinoid agents in animals. Crit Rev Neurobiol 13(3):243–281

Chatterjee N, Callen S, Seigel GM, Buch SJ (2011) HIV-1 Tat-mediated neurotoxicity in retinal cells. J Neuroimmune Pharmacol 6(3):399–408

Chen J, Matias I, Dinh T, Lu T, Venezia S, Nieves A, Woodward DF, Di Marzo V (2005) Finding of endocannabinoids in human eye tissues: implications for glaucoma. Biochem Biophys Res Commun. 330(4):1062–1067

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258(5090):1946–1949

Dhawan N, Puangco J, Jandial R (2008) In search of a treatment for Alzheimer's disease and potential immunonosuppresive therapeutic interventions. Neuro Endocrinol Lett 29(4):410–420

Di Marzo V (2018) New approaches and challenges to targeting the endocannabinoid system. Nat Rev Drug Discov 17:623–639

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC et al (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372:686–691

Di Marzo V, Melck D, Bisogno T, De Petrocellis L (1998) Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci 21(12):521–528

Downer EJ, Clifford E, Gran B, Nel HJ, Fallon PG, Moynagh PN (2011) Identification of the synthetic cannabinoid R(+)WIN55,212-2 as a novel regulator of IFN regulatory factor 3 activation and IFN-beta expression: relevance to therapeutic effects in models of multiple sclerosis. J Biol Chem 286(12):10,316–10,328

Eljaschewitsch E, Witting A, Mawrin C, Lee T, Schmidt PM, Wolf S, Hoertnagl H, Raine CS, Schneider-Stock R, Nitsch R, Ullrich O (2006) The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. Neuron 49(1): 67–79

El-Remessy AB, Khalil IE, Matragoon S et al (2003) Neuroprotective effect of (−)Δ9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am J Pathol 163(5):1997–2008

Fan SF, Yazulla S (2003) Biphasic modulation of voltage-dependent currents of retinal cones by cannabinoid CB1 receptor agonist WIN 55212-2. Vis Neurosci 20(2):177–188

Forest DL, Johnson LV, Clegg DO (2015) Cellular models and therapies for age-related macular degeneration. Dis Model Mech 8(5):421–427

Fowler CJ, Holt S, Nilsson O, Jonsson KO, Tiger G, Jacobsson SO (2005) The endocannabinoid signaling system: pharmacological and therapeutic aspects. Pharmacol Biochem Behav 81(2): 248–262

Garcia-Ovejero D, Arevalo-Martin A, Petrosino S, Docagne F, Hagen C, Bisogno T, Watanabe M, Guaza C, Di Marzo V, Molina-Holgado E (2009) The endocannabinoid system is modulated in response to spinal cord injury in rats. Neurobiol Dis 33(1):57–71

Hillard CJ (2008) Role of cannabinoids and endocannabinoids in cerebral ischemia. Curr Pharm Des 14(23):2347–2361

Hind WH, Tufarelli C, Neophytou M, Anderson SI, England TJ, O'Sullivan SE (2015) Endocannabinoids modulate human blood-brain barrier permeability in vitro. Br J Pharmacol 172(12):3015–3027

Hirotani T, Lee PY, Kuwata H, Yamamoto M, Matsumoto M, Kawase I, Akira S, Takeda K (2005) The nuclear I kappaB protein I kappa BNS selectively inhibits lipopolysaccharide-induced IL-6 production in macrophages of the colonic lamina propria. J Immunol 174:3650–3657

Hofman FM, Hinton DR (1992) Tumor necrosis factor-alpha in the retina in acquired immune deficiency syndrome. Invest Ophthalmol Vis Sci 33(6):1829–1835

Ilyasov AA, Milligan CE, Pharr EP, Howlett AC (2018) The endocannabinoid system and oligodendrocytes in health and disease. Front Neurosci. 12(733)

Ishikawa T, Yoshitomi C, Zorumski F, Izumi Y (2015) Experimentally induced mammalian models of glaucoma. BioMed Res Int 2015:281214

Järvinen T, Pate DW, Laine K (2002) Cannabinoids in the treatment of glaucoma. Pharmacol Ther 95(2):203–220

Kallendrusch S, Hobusch C, Ehrlich A, Ziebell S, Ueda N, Geisslinger G, Koch M, Dehghani F (2012) Site-specific and time-dependent activation of the endocannabinoid system after transection of long-range projections. PLoS One 7(3):e33537

Kaur I, Behl T, Bungau S, Zengin G, Kumar A, El-Esawi MA, Khullar G, Venkatachalam T, Arora S (2020) The endocannabinoid signaling pathway as an emerging target in pharmacotherapy, earmarking mitigation of destructive events in rheumatoid arthritis. Life Sci 257:118,109

Krishnan G, Chatterjee N (2012) Endocannabinoids alleviate proinflammatory conditions by modulating innate immune response in muller glia during inflammation. Glia 60(11):1629–1645

Krishnan G, Chatterjee N (2014) Endocannabinoids affect innate immunity of Muller glia during HIV-1 Tat cytotoxicity. Mol Cell Neurosci 59:10–23

Krishnan G, Chatterjee N (2015a) Anandamide rescues retinal barrier properties in Müller glia through nitric oxide regulation. Neuroscience. 284:536–545

Krishnan G, Chatterjee N (2015b) Differential immune mechanism to HIV-1 Tat variants and its regulation by AEA [corrected]. Sci Rep 5:9887. Erratum in: Sci Rep. 5:12206.

Kumar A, Pandey RK, Miller LJ, Singh PK, Kanwar M (2013) Muller glia in retinal innate immunity: a perspective on their roles in endophthalmitis. Crit Rev Immunol 33(2):119–135

Laine K, Järvinen T, Savinainen J, Laitinen JT, Pate DW, Järvinen K (2001) Effects of topical anandamide-transport inhibitors, AM404 and olvanil, on intraocular pressure in normotensive rabbits. Pharm Res 18(4):494–499

Laine K, Järvinen K, Mechoulam R, Breuer A, Järvinen T (2002) Comparison of the enzymatic stability and intraocular pressure effects of 2-arachidonylglycerol and noladin ether, a novel putative endocannabinoid. Invest Ophthalmol Vis Sci 43(10):3216–3222

Laing JG, Koval M, Steinberg TH (2005) Association with ZO-1 correlates with plasma membrane partitioning in truncated connexin45 mutants. J Membr Biol 207(1):45–53

Lambert DM, Fowler CJ (2005) The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. J Med Chem 48(16):5059–5087

Lax P, Esquiva G, Altavilla C, Cuenca N (2014) Neuroprotective effects of the cannabinoid agonist HU210 on retinal degeneration. Exp Eye Res 120:175–185

Maccarrone M, Bab I, Bíró T, Cabral GA, Dey SK, Di Marzo V, Konje JC, Kunos G, Mechoulam R, Pacher P, Sharkey KA, Zimmer A (2015) Endocannabinoid signaling at the periphery: 50 years after THC. Trends Pharmacol Sci 36(5):277–296

Matias J, Wang A, Moriello S, Nieves A, Woodward DF, Di Marzo V (2006) Changes in endocannabinoid and palmitoylethanolamide levels in eye tissues of patients with diabetic retinopathy and age-related macular degeneration. Prostaglandins Leukotrienes Essential Fatty Acids 75(6):413–418

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR et al (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50(1):83–90

Middleton TP, Protti DA (2011) Cannabinoids modulate spontaneous synaptic activity in retinal ganglion cells. Vis Neurosci 28(5):393–402

Naccarato M, Pizzuti D, Petrosino S, Simonetto M, Ferigo L, Grandi FC, Pizzolato G, Di Marzo V (2010) Possible anandamide and palmitoylethanolamide involvement in human stroke. Lipids Health Dis 9:47

Papa SM (2008) The cannabinoid system in Parkinson's disease: multiple targets to motor effects. Exp Neurol 211(2):334–338

Piro JR, Suidan GL, Quan J, Pi Y, O'Neill SM, Ilardi M, Pozdnyakov N, Lanz TA, Xi H, Bell RD, Samad TA (2018) Inhibition of 2-AG hydrolysis differentially regulates blood brain barrier permeability after injury. J Neuroinflammation 15(1):142

Pryce G, Ahmed Z, Hankey DJ, Jackson SJ, Croxford JL, Pocock JM, Ledent C, Petzold A, Thompson AJ, Giovannoni G, Cuzner ML, Baker D (2003) Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. Brain 126(Pt 10):2191–2202

Rockwell CE, Kaminski NE (2004) A cyclooxygenase metabolite of anandamide causes inhibition of interleukin-2 secretion in murine splenocytes. J Pharmacol Exp Ther 311(2):683–690

da Rovare VP, Magalhães GPA, Jardini GDA, Beraldo ML, Gameiro MO, Agarwal A, Luvizutto GJ, Paula-Ramos L, Camargo SEA, de Oliveira LD, Bazan R, El Dib R (2017) Cannabinoids for spasticity due to multiple sclerosis or paraplegia: a systematic review and meta-analysis of randomized clinical trials. Complement Ther Med 34:170–185

Sawada M, Suzumura A, Yamamoto H, Marunouchi T (1990) Activation and proliferation of the isolated microglia by colony stimulating factor-1 and possible involvement of protein kinase C. Brain Res 509(1):119–124

Schlicker E, Timm J, Göthert M (1996) Cannabinoid receptor-mediated inhibition of dopamine release in the retina. Naunyn Schmiedebergs. Arch Pharmacol 354(6):791–795

Schwitzer T, Schwan R, Angioi-Duprez K, Giersch A, Laprevote V (2016) The endocannabinoid system in the retina: from physiology to practical and therapeutic applications. Neural Plast 2016:2916732

Scotter EL, Goodfellow CE, Graham ES, Dragunow M, Glass M (2010) Neuroprotective potential of CB1 receptor agonists in an in vitro model of Huntington's disease. Br J Pharmacol 160(3): 747–761

Shamsuddin N, Kumar A (2011) TLR2 mediates the innate response of retinal Muller glia to Staphylococcus aureus. J Immunol 186(12):7089–7097

Stamer WD, Golightly SF, Hosohata Y, Ryan EP, Porter AC, Varga E, Noecker RJ, Felder CC, Yamamura HI (2001) Cannabinoid CB(1) receptor expression, activation and detection of endogenous ligand in trabecular meshwork and ciliary process tissues. Eur J Pharmacol 431(3):277–286

Straiker A, Sullivan JM (2003) Cannabinoid receptor activation differentially modulates ion channels in photoreceptors of the tiger salamander. J Neurophysiol 89(5):2647–2654

Straiker A, Stella N, Piomelli D, Mackie K, Karten HJ, Maguire G (1999) Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. Proc Natl Acad Sci U S A 96(25):14,565–14,570

Tanaka M, Sackett S, Zhang Y (2020) Endocannabinoid modulation of microglial phenotypes in neuropathology. Front Neurol 11:87

Thiel VE, Audus KL (2001) Nitric oxide and blood-brain barrier integrity. Antioxid Redox Signal 3(2):273–278

Walter L, Franklin A, Witting A, Wade C, Xie Y, Kunos G, Mackie K, Stella N (2003) Nonpsychotropic cannabinoid receptors regulate microglial cell migration. J Neurosci 23(4): 1398–1405

Wei Y, Wang X, Zhao F, Zhao PQ, Kan XL (2013) Cannabinoid receptor 1 blockade protects human retinal pigment epithelial cells from oxidative injury. Mol Vis 19:357–366

Witting A, Chen L, Cudaback E, Straiker A, Walter L, Rickman B, Möller T, Brosnan C, Stella N (2006) Experimental autoimmune encephalomyelitis disrupts endocannabinoid-mediated neuroprotection. Proc Natl Acad Sci U S A 103(16):6362–6367

Yasmin F, Colangeli R, Morena M, Filipski S, van der Stelt M, Pittman QJ, Hillard CJ, Teskey GC, McEwen BS, Hill MN, Chattarji S (2020) Stress-induced modulation of endocannabinoid signaling leads to delayed strengthening of synaptic connectivity in the amygdala. Proc Natl Acad Sci U S A 117(1):650–655

Yazulla S (2008) Endocannabinoids in the retina: from marijuana to neuroprotection. Prog Retin Eye Res 27(5):501–526

Yazulla S, Studholme KM (2004) Vanilloid receptor like 1 (VRL1) immunoreactivity in mammalian retina: colocalization with somatostatin and purinergic P2X1 receptors. J Comp Neurol 474(3):407–418

Zhang T, Su J, Guo B, Zhu T, Wang K, Li X (2014) Ursolic acid alleviates early brain injury after experimental subarachnoid hemorrhage by suppressing TLR4-mediated inflammatory pathway. Int Immunopharmacol 23(2):585–591

Drosophila melanogaster: An Immaculate Model for Glial Research

Pragati, Shweta Tandon, Aqsa, Prerna Aggarwal, and Surajit Sarkar

Abstract

Although glial cells have been typically regarded as support cells of the neurons, it is increasingly clear now that they play a critical role(s) in the development, function, and maintenance of the nervous system and are also required for the maintenance of ionic balance in the CNS, synaptic signaling, regulation of the blood-brain barrier, and brain immune response. However, several functional aspects of glial cells remain enigmatic; for instance, their presence and potential function(s) in glutamatergic, cholinergic or GABAergic synapses are largely unknown. Similarly, their precise contribution in the etiology of neuronal disorders is still elusive. In fact, mammalian glia are difficult to analyze in vivo, and our understanding of glia biology has largely emerged from the studies on the primary cultures. Nevertheless, the majority of such in vitro findings have not been confirmed or repeated in experiments with the living organism, which is important since glia and neurons exist in close association with each other soon after the differentiation. In view of the above, and also due to limitations attached with the studies on human genetics, *Drosophila* has emerged as one of the prime model systems for glial research. Intriguingly, despite the significant difference in size, the *Drosophila* adult brain exhibits structures and functions similar to the mammalian brains and shares a number of glial characteristics. In view of the availability of sophisticated genetic tools, diverse behavioral features, and evolutionarily conserved genome, the *Drosophila* glia seem well-positioned to provide exciting insights into glial biology, which will be relevant to the glial functions in higher organisms including humans. The present chapter summarizes the current knowledge and enduring contribution of *Drosophila* in glia research.

Pragati · S. Tandon · Aqsa · P. Aggarwal · S. Sarkar (✉)
Department of Genetics, University of Delhi South Campus, New Delhi, India
e-mail: sarkar@south.du.ac.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_26

Keywords

Drosophila · Brain · Glia · Neurodegeneration

1 Introduction

Among the important characteristics, movement/motion is one of the most fascinating properties of the members of the animal kingdom which helps in sensing the external stimuli and responding correspondingly in a different situation. To enable this, a well-established neuronal network is required in controlling movement and adaptation of other equivalent behavioral responses (Bittern et al. 2020). The central nervous system (CNS) is one such evolutionarily developed system in the animal kingdom which helps in computing the sensory signals to motor program regulating muscular movements (Collet et al. 2013). To achieve this, neurons communicate with each other via synapses with the assistance of electrical impulses or action potential. The action potentials are generated by voltage-gated ion channels, and neurons respond to change in their membrane potential by releasing the neurotransmitters (Bittern et al. 2020).

In addition to the different types of neuronal cells which make a significant constituent of CNS, they are not the sole cell type(s) which establishes the nervous system. The glial cells, which were originally thought of as a simple support to neuronal cells, have now emerged as equally important cell type(s) of the nervous system (Jäkel and Dimou 2017). Although not capable of inducing a change in membrane potential as fast as neuronal cells, the glial cells exist in close association with the neurons, and in some parts of the mammalian brain, they even outnumber the neuronal cells (Herculano-Houzel 2014). First documented by Rudolph Virchow in 1858, two important roles were suggested for the glial cells – i.e., providing mechanical support and tissue repair (Virchow 1856, 1858). Highlighted as supporting cells of the nervous system, they were referred to as "nerve glue"/ "nervenkitt" (the German word for nerve glue) and subsequently named as "glial cell" which is an English translation of Greek word *glía* denoting "glue."

The glial cells perform various disparate roles in the nervous system, for instance, regulating the proliferation of neuronal stem cells (Ebens et al. 1993), wrapping of the nerves and every discrete axon (Barres 2008; Nave and Trapp 2008), removal of eradicated neurons and debris during development through phagocytosis (Sonnenfeld and Jacobs 1995; Freeman et al. 2003; Awasaki and Ito 2004; Watts et al. 2004), maturation and development of synapses (Barres 2008), etc. Further, in a mature nervous system, the glial cells also help in isolating and protecting neurons by forming/controlling the blood-brain barrier (Abbott 2005), maintaining ionic balance in the CNS (Danbolt 2001), and acting as major immune cells (Bittern et al. 2020). However, the above list is just a partial representation of the true array of the glial roles in a well-matured CNS.

In spite of having a prevalent role of glial cells in the nervous system, the molecular pathways which facilitate adopting glial functioning are not fully

understood. For instance, it is increasingly known that glial cells surround and ensheath each synapse, but it is unclear what drives the glial cells to wrap around the synapses. Do the glial cells help in passing on the information via synapse to other cellular axons? Also, due to the involvement of glutamatergic/GABAergic/cholinergic neurons in the pathogenesis of various neurodegenerative diseases, it is of utmost importance to unravel the in vivo role of the glial cells which surround the synapses of these respective neurons, to establish the contribution of glial cells in disease etiology (Stork et al. 2012).

Interestingly, some contemporary studies suggest an imperative role(s) of glial cells in aging and pathogenesis of neurodegenerative diseases. With aging, a decline in the activities of microglia, its migration, and chemotaxis has been noted (Spittau 2017). For example, the capacity of microglia to engulf the neurodegenerative disease-causing protein aggregates such as amyloid-beta (Aβ) (Floden and Combs 2011) or alpha-synuclein (α-syn) (Bliederhaeuser et al. 2016) deteriorates in mammals. Also, stimulated activity of microglia and neuroinflammatory profiles have been observed in various neurodegenerative diseases such as Huntington's (Crotti et al. 2014), Alzheimer's (Lopategui Cabezas et al. 2014; Jones and Kounatidis 2017), and Parkinson's disease (Nolan et al. 2013; Taylor et al. 2013).

To unravel the functional relevance of the glial cells, it is important to generate an in-depth understanding of the molecular pathways that drive the glia-neuron interaction. The most difficult part of studying glial biology is to analyze the mammalian glia in vivo due to the scarcity of markers/tools which help to visualize and manipulate the glial cells. Though the primary tissue cultures have emerged as a handy tool to study the glia-neuron interaction, however, the majority of observations from such in vitro studies could not be replicated in the animal models (Stork et al. 2012). This might be due to the changes developed in glial morphology as they get dissociated from an in vivo environment onto an in vitro plate setup. Thus, observations inferred from in vitro arrangements should be essentially verified using an in vivo model system.

Drosophila melanogaster, already an established model organism, can serve as an exceptional system to study the in-depths of glia-neuron crosstalk in vivo. Due to the availability of the powerful genetic tools in fly, it is relatively convenient to investigate the complex molecular details to decipher the fundamental questions related to glial biology. This chapter attempts to provide an overview of the flexible genetic tools available with *Drosophila*, a brief account of fly gliogenesis, glial cell types, and their functions, and also provides an account of some recent discoveries about the involvement of glial cells in etiology of various human neurodegenerative disorders.

2 *Drosophila*: A Prime Model for Glia Research

Commonly known as "fruit fly," *D. melanogaster* is one of the most studied organisms in biology to comprehend the fundamentals of genetics and development. This tiny invertebrate system has been used extensively in biomedical research to

address various aspects of life such as aging, neurodegeneration, inflammation, immunity, circadian rhythms, etc. Basically, *Drosophila* emerged as a prime research model due to its ease of culturing and capacity to maintain homogeneous populations, short recordable life span, high fecundity, large collection of mutant/insertion/transgenic lines, availability of the balancer chromosomes, and obtainability of well-annotated complete genome sequence. Also, *Drosophila* offers powerful genetic tools such as P-element mutagenesis, *UAS-Gal4/Gal80* system (to achieve tissue-specific/ectopic expression or downregulation of a gene of interest), *FLP-FRT* (flippase-flippase recognition target; facilitates site-specific recombination), *FLP*-out and *MARCM* (mosaic analysis with a repressible cellular marker; both the techniques have been utilized to study the morphology of individual cells or a subset of Gal4-positive cells), CRISPR-Cas9-based precise genome editing, etc. (Brand and Perrimon 1993; Theodosiou and Xu 1998; Lee and Luo 1999; Wong et al. 2002; Awasaki and Lee 2011; Gratz et al. 2015). Henceforth, due to the vast availability of diverse genetic toolkit, the fly genome is highly approachable and easy to manipulate, providing a robust handle to study a variety of intracellular and intercellular molecular interactions. Interestingly, all these advantages helped to overcome the limitations faced while studying glia-neuron relationship, which are still tenacious in the higher mammalian systems.

Drosophila undergoes holometabolous metamorphosis, exhibiting distinct developmental stages. Each of these stages offers a different microenvironment and arrangement of cells that can be well-utilized to address different aspects of glial biology. In spite of being an invertebrate, *Drosophila* acquires a fairly complex nervous system in a brief developmental time period in which the relative distribution of neuron to glia has been found to be ~90% to ~10% (Freeman 2015; Kremer et al. 2017; Crews 2019). There are about 350 neurons and 30 glial cells present in each abdominal hemineuromere of embryonic CNS, with approximately eight to ten peripheral glia (Technau et al. 2006). Due to a limited number of neuronal and glial cells residing in each segment of the embryonic nervous system, as well as their peculiar arrangement and expression of specific molecular markers, *Drosophila* embryo makes an excellent system to investigate the role of glial cells in early events of neurogenesis. The number of these cells expands as the organism progresses toward the larval stage endowing it with complex behavior such as foraging, crawling, etc.

The larval CNS harbors nearly 15,000 cells with a fraction of 1000 glial cells among them (Monedero Cobeta et al. 2017). Post metamorphosis, the structures mature to form a more complex organ consisting of ~1,50,000 neurons and ~15,700 glial cells (Jenett et al. 2012; Kremer et al. 2017). With the advent of a well-annotated and affordable number of genetic tools as noted earlier, and the availability of Gal4 driver lines as enlisted in Table 26.1, it is possible to mark and study various glial cell types during the developmental processes and also in adults (Awasaki et al. 2008; Stork et al. 2008; Doherty et al. 2009). In addition, several *Drosophila*-specific categorized markers are available which could be used in tracing the glial cell subpopulations (Ito et al. 1995; Beckervordersandforth et al. 2008; von Hilchen et al. 2008). Most of these markers are very useful in localizing

Table 26.1 List of available Gal4 driver lines for manipulation of gene expression in *Drosophila* glial cells

S. no.	Gal4 driver line	Expression pattern	References
1.	*repo-Gal4*	All glia except midline	Sepp et al. (2001)
2.	*repo-Gal4-4.3*	All glia except midline	Lee and Jones (2005)
3.	*repo:LexA::GAD*	All glia except midline	Lai and Lee (2006)
4.	*gcm-Gal4*	All glia (except midline), apodemal cells, and macrophages	Paladi and Tepass (2004)
5.	*htl-Gal4*	Longitudinal glia and other glia	Shishido et al. (1997)
6.	*rl82-Gal4 (gliotactin-Gal4)*	Pronounced in subperineurial glia, weaker in perineurial glia	Sepp and Auld (1999)
7.	*Spg-Gal4*	Subperineurial glia	Stork et al. (2008), Mayer et al. (2009)
8.	*alrm-Gal4*	Astrocyte-like glia	Doherty et al. (2009)
9.	*deaat1-Gal4*	Astrocyte-like glia, some cortex, weak in neurons	Rival et al. (2004, 2006)
10.	*Mz709-Gal4*	Ensheathing glia, neurons	Ito et al. (1995)
11.	*nrv2-Gal4*	Cortex, subperineurial, ensheathing glia, and wrapping glia of abdominal nerves, and weakly expresses in astrocyte-like glia	Sun et al. (1999)
12.	*moody-Gal4*	Subperineurial glia	Schwabe et al. (2005)
13.	*NP6293-Gal4*	Perineurial glia, subset neurons	Hayashi et al. (2002), Awasaki et al. (2008)
14.	*NP2276-Gal4*	Subperineurial glia	Hayashi et al. (2002), Awasaki et al. (2008)
15.	*NP577-Gal4*	Cortex glia	Hayashi et al. (2002), Awasaki et al. (2008)
16.	*NP2222-Gal4*	Cortex glia	Hayashi et al. (2002), Awasaki et al. (2008)
17.	*NP3233-Gal4*	Astrocyte-like glia	Hayashi et al. (2002), Awasaki et al. (2008)
18.	*NP1243-Gal4*	Astrocyte-like glia and weaker in ensheathing glia and cortex glia	Hayashi et al. (2002), Awasaki et al. (2008)
19.	*NP6520-Gal4*	Ensheathing glia and weaker in cortex glia	Awasaki et al. (2008)
20.	*Tret1-1-Gal4*	Perineurial glia of the CNS	Volkenhoff et al. (2015)

(continued)

Table 26.1 (continued)

S. no.	Gal4 driver line	Expression pattern	References
21.	*Gli-Gal4*	Weak driver of subperineurial glia	Auld et al. (1995)
22.	*R55B12-Gal4*	Cortex glia	Li et al. (2014)
23.	*R83E12-Gal4*	Ensheathing glia	Li et al. (2014)
24.	*R74E02-Gal4*	Wrapping glia of the eye disc	Li et al. (2014)
25.	*R95A08-Gal4*	Wrapping glia of the eye disc	Li et al. (2014)
26.	*R85G01-Gal4*	Perineurial glia of the entire CNS	Kremer et al. (2017)
27.	*R54C07-Gal4*	Subperineurial glia of the entire CNS	Kremer et al. (2017)
28.	*R54H02-Gal4*	Cortex glia in CNS except lamina	Kremer et al. (2017)
29.	*R86E01-Gal4*	Astrocyte-like glia in lamina	Kremer et al. (2017)
30.	*R56F03-Gal4*	Ensheathing glia of the neuropile	Kremer et al. (2017)
31.	*R75H03-Gal4*	Ensheathing glia of the tracts	Kremer et al. (2017)

the glial cell population at the embryonic stage, though they are somewhat poorly adaptable for later developmental stages. The protein markers which are available for tracing the glial cell populations at later stages are not well-characterized yet for their morphological and molecular identity (Stork et al. 2012). Nevertheless, many of these protein markers aid in visualizing the various glial population in vivo as well as in examining the manifestation on the nervous system when the genetics of glial cells are manipulated (Awasaki and Lee 2011; Stork et al. 2012; Kremer et al. 2017). Table 26.2 enlists several molecular markers corresponding to various glial cells present in the *Drosophila* nervous system.

To provide a basic understanding of how different *Drosophila* glial cells are originated/developed from their progenitor cells, and what all signaling cascades guide their different cellular fates and functions, the next section of this chapter offers a brief overview of the process of glial cell formation or "gliogenesis" in *Drosophila*.

3 Gliogenesis in *Drosophila*

The process by which different glial cells such as midline glia, cortex glia, surface glia, and neuropil-associated glia are generated from their respective progenitor cells and subsequently develop into mature glial cells is known as gliogenesis. The *gcm*

Table 26.2 Some commonly used molecular marker/antibodies to visualize specific glial subtypes in *Drosophila*

S. no.	Markers/ antibodies	Expression pattern	References
1.	Anti-Repo	All glial cells (except midline)	Campbell et al. (1994)
2.	Anti-Gcm	All glial cells at early stages (except midline)	Alfonso and Jones (2002)
3.	Anti-Moody-β	Subperineurial glia	Bainton et al. (2005)
4.	Anti-Moody-α	Subperineurial glia	Bainton et al. (2005)
5.	Anti-Gs2	Subset of longitudinal glia	Thomas and van Meyel (2007)
6.	Anti-NrxIV	Subperineurial glia, neurons, epithelia	Baumgartner et al. (1996)
7.	Anti-Tret1-1	Perineurial glia of the CNS	Volkenhoff et al. (2015)
8.	Anti-Apontic	Perineurial glia of the PNS	Eulenberg and Schuh (1997)
9.	Anti-MDR65	Subperineurial glia	Mayer et al. (2009)
10.	Anti-PointedP2	Cortex glia	Avet-Rochex et al. (2012)
11.	Anti-Ebony	Astroglia-like glia	Suh and Jackson (2007)
12.	Anti-EAAT1	Astroglia-like glia	Peco et al. (2016)
13.	Anti-GAT	Astroglia-like glia	Stork et al. (2014)
14.	Anti-Cut	Wrapping glial cells of abdominal nerves and eye discs, weak expression in subperineurial glia	Bauke et al. (2015)

(glial cell missing) gene, which encodes for a transcription factor, is essentially required for determining the fate of a cell to become a glial cell (Hosoya et al. 1995; Jones et al. 1995; Vincent et al. 1996). As the name suggests, loss of function mutation in *gcm* prevents the formation of glial cells, whereas its ectopic expression in the CNS drives the presumptive neuronal cells to become the glial cells instead. Therefore, *gcm* expression is needed for the formation of glial cells, but its repression leads to the formation of neuronal cells (Altenhein et al. 2016). The promoter region of the *gcm* gene possesses some cis-controlling elements which control its expression and autoregulation. The sequences between 200 bp and 1.4 kb upstream of promoter provide a broad but weaker pan-neuronal expression; however, the upstream sequences between 1.4 and 4.4 kb not only control the lineage-specific expression but also act as repressor sequences. Further, some other lineage-specific elements have been suggested to be present downstream to the gene and also as far as 18 kb upstream of the promoter (Jones et al. 2004).

Interestingly, in addition to the above noted mode of regulations, the Gcm protein harbors DNA binding sites referred to as "Gcm binding sites" (GBSs), which bind to the DNA sequence located between 4.4 and 7.4 kb upstream to the promoter and facilitate autoregulation of *gcm* expression (Jones et al. 2004). Despite being a master regulator of the glial cell differentiation, *gcm* functions along with its homolog *gcm2* for plasmocyte/macrophage lineage of hemocytes (Alfonso and Jones 2002; Bernardoni et al. 1997; Lebestky et al. 2000). Intriguingly, these two genes jointly control hemocyte migration and their development into active macrophages (Alfonso and Jones 2002). The tightly regulated promoter of *gcm* which governs the dual events, i.e., blood and glial cell development, indicates toward the possibility of the involvement of additional transcription factors, which might be working along with Gcm to control glial development in *Drosophila*. The interplay between the other transcription factors and *gcm*, which regulates the process of gliogenesis, has been briefly described below.

It has been suggested that in consort with Gcm, other transcription factors such as *reversed polarity* (*repo*), *pointed* (*pnt*), and *tramtrack* (*ttk*), along with the other glial differentiation genes, facilitate glial development (Lee and Jones 2005). All these genes are downstream targets of *gcm*, and a threshold level of Gcm protein is required to initiate their expression. For instance, an increasing level of Gcm activates the expression of one of its downstream gene *repo*. Initially, Repo also acts as a positive regulator for *gcm* and helps in maintaining its expression in the early stages of development, until the glial fate and differentiation occur (Flici et al. 2014). However, the increasing levels of Repo alter the molecular ratio between the two proteins and their absolute levels and then drive Repo to send inhibitory signals to *gcm*, thus exerting a negative feedback loop on its expression (Flici et al. 2014). Hence, a loop is formed between Gcm and Repo in order to maintain their relative optimum levels in the cell. Further, the expression of *pnt* and *ttk* also depends on the expression status of *gcm*. The expression of Pnt positively promotes the glial cell differentiation, whereas *ttk* ensures the glial development by repressing the neuronal fate (Lee and Jones 2005). Taken together, *gcm* facilitates glial development by persuading the expression of *repo* and *pnt* and by suppressing the induction of neuronal fate via activated *ttk*.

Furthermore, Pnt and Repo both function together to regulate other downstream genes such as locomotion defects (*loco*), which are known to be involved in axonal ensheathment and glial-glial connections (Klaes et al. 1994; Granderath et al. 2000). Interestingly, several other genes that are involved in controlling glial differentiation, axon ensheathment, neurotransmitter metabolism, phagocytosis, and migration exhibit Gcm binding sites near the promoter region, highlighting the master regulatory role of Gcm in glial cell development (Freeman et al. 2003; Altenhein et al. 2006). To provide a basic outline of the development of different glial cell types, a brief account of glial cellular lineages has been provided in the subsequent texts.

3.1 Different Glial Lineages in *Drosophila*

Although all the embryonic glial (expect midline glia) cells are derived from a master cell expressing *gcm*, they are however generated in diverse ways from a modest number of progenitors (Soustelle and Giangrande 2007). Six different lineage types have been identified which can give rise to all kinds of embryonic glia cells (Altenhein et al. 2016). The primitive precursor cell which gives rise to the most glial cells is referred to as neuroglioblast (NGB). Interestingly, in addition to the glial cells, some neuroglioblasts may give rise to the neuronal cells as well. There are three different cellular lineages known where neuroglioblasts divide and give rise to a neuronal or a glial cell. The type 1 lineage comprises of three neuroglioblasts which divide into a cell with stem cell mode (neuroglioblast only) and another cell called ganglion mother cell (GMC) which further divides asymmetrically to give rise to a glial and neuronal cell. The type 2 comprises of three neuroglioblasts with two subtype cellular lineages, i.e., type 2a and type2b. The former is like type 1 lineage which gives rise to both neuronal and ganglion mother cells, while the latter population of neuroglioblasts gives rise to neuroglioblast with ganglion mother cells that form two neuronal daughter cells. Interestingly, due to some molecular cues received, neuroglioblasts of both the lineages can switch their fate to glioblast that can give rise to only two daughter glial cells. Type 2 lineage can generate a variety of glial cell types. For instance, NB7-4 can give rise to cell body glia, surface glia, and neuropil glia (Beckervordersandforth et al. 2008). Lastly, type 3 includes two subtypes, i.e., type 3a and type 3b. In type 3a lineage, neuroglioblast divides to gives rise to one glioblast and another neuroblast that subsequently generates glial and neuronal cells, respectively. Interestingly, the type 3b lineage comprises of only glioblast that can produce glial cells only. Type 3a (NB6-4) forms cell body glia while type 3b forms neuropil-associated glia/longitudinal glia (Altenhein 2015). Figure 26.1 provides a schematic representation of various cellular lineages differentiating from their precursor cells.

As described above, neuroblasts of type 2a lineage can divide and give rise to either combination of neurons and glia, or due to some molecular cues received, it can change into glia producing cells. Similarly, neurons producing type 2b cells can also change their fate to glial producing cells (Fig. 26.1). This complex regulation is maintained by the interplay of different transcription factors/genes, some of which are described in the following section.

3.2 Establishment of Glial Cell Fate

The advancement of embryonic CNS in *Drosophila* occurs from 30 distinguished stem cells known as neuroblasts, found in each hemisegment with neurons and glia produced in a stereotyped pattern (Goodman and Doe 1993). As mentioned above, neuroglioblasts are the ones that can produce both the neurons and glial cells (Udolph et al. 1993; Bossing et al. 1996; Schmidt et al. 1997). However, the cellular and molecular details on how a single stem cell is capable of producing alternative

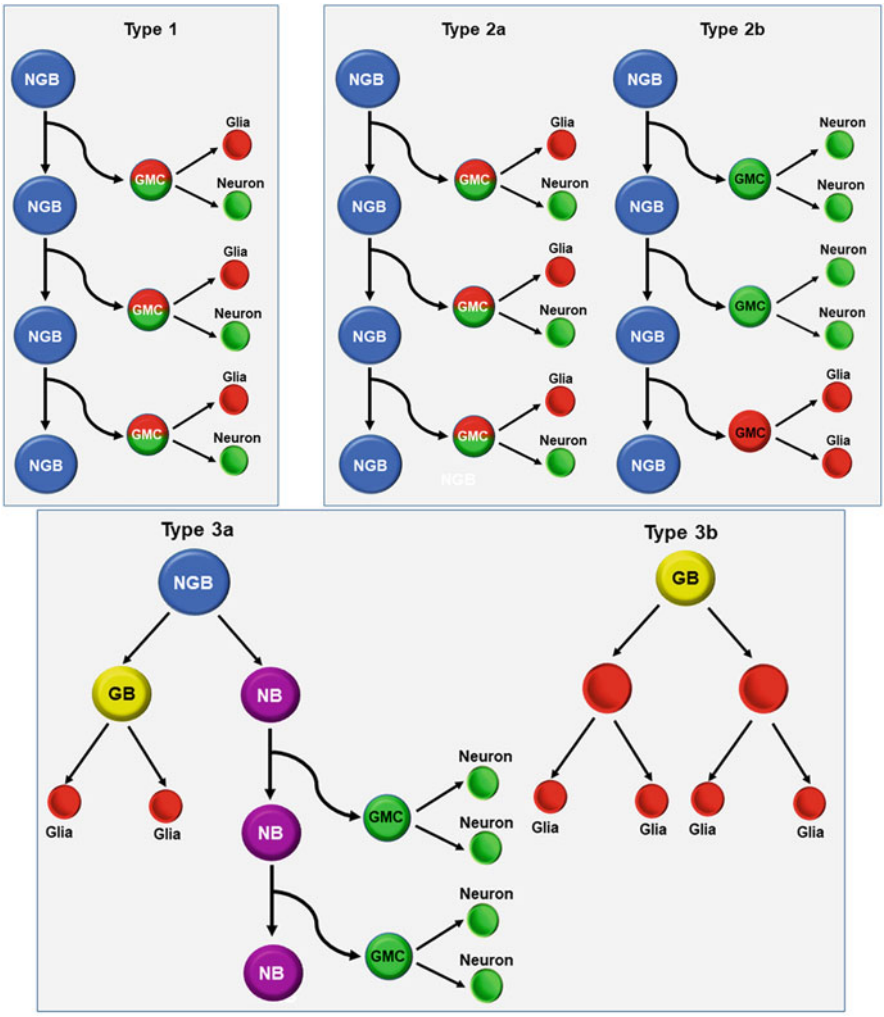

Fig. 26.1 Schematic showing diverse possible ways of glial precursor division in *Drosophila* Neuroglioblasts (NGBs) divide into neuronal and glial cells, while neuroblasts (NBs) and glioblasts (GBs) give rise to only neuronal and glial cells, respectively. The division pattern is of three types – type 1, type 2, and type 3 – where the precursor NGBs divide into ganglion mother cell (GMC) which either gives rise to both glial and neuronal cells (type 1 and type 2a) or can switch their fate to glial/neuronal producing cells (type 2b). However, in type 3 cells, from initial division separate fates can be maintained of GBs or NBs cells (type 3a and type 3b)

fates are still inadequate. Nevertheless, a key protein Gcm has emerged as a key regulator of the noted capability of neuroglioblasts (Freeman and Doe 2001). The Gcm protein localizes in the neuroglioblast before division and is also maintained in both neuroblast and glioblast after the division. Later, the levels of Gcm protein are abrogated in presumptive neuron forming cell and upregulated in the presumptive

glial cell. The equally distributed *gcm* transcripts get upregulated in the presumptive glial cell by *prospero* (*pros*) which is present asymmetrically to presumptive glial cells (Freeman and Doe 2001). Another important gene *miranda* (*mira*) aids in achieving the unequal distribution of *pros.* Moreover, the Mira protein itself localizes asymmetrically and regulates the cellular distribution of *pros*, which further assists in determining the glial cell fate. Interestingly, a mutation in *mira* disrupts the asymmetric distribution of *pros* RNA and protein which subsequently influences the fate of presumptive neuroblasts in some hemisegments (Freeman and Doe 2001).

Interestingly, a neuroglioblast NGB6-4T/NB6-4T in the thoracic region can give rise to neuroblast and glioblast by asymmetric division, whereas it's homologous neuroblast in the abdominal region NB6-4A, and a glioblast GB6-4A, from a type 3b lineage, can only give rise to glial cells (Berger et al. 2005; Crews 2019). This difference between type 3a and type3b lineage is determined by several genes such as *CycE*, *apontic (apt)*, and *Hox* which have been shown to regulate differential fate between these neuroglioblasts (Crews 2019). NGB6-4T can give rise to both neuronal and glial cell types due to asymmetric distribution of transcription factors, i.e., *gcm* and *pros*, whereas the symmetric distribution of these two factors in NB6-4A generates only glial cells (Freeman and Doe 2001). In the above context, a G1 cyclin gene *dmCycE* (*CycE*) has been found to be playing a very important role. The *CycE* functions upstream of *pros* and *gcm* for neuronal sublineage specification. In NB6-4T, *CycE* is localized specifically to cells with neuronal fate and prevents the localization of *pros* and *gcm* (Berger et al. 2005). Another important gene that aids in regulating the differential determination of neuronal and glial fate is *apontic* (*apt*). The *apt* gene product in NB6-4T cells induces the production of *CycE*, thus resulting in the deterioration of *pros* and *gcm* (Berger et al. 2005; Shen et al. 2018). However, *gcm* in NB6-4A suppresses *apt* and *CycE* expression, thereby supporting the attainment of glia fates (Shen et al. 2018). The NB6-4A maintains the glial fate from the inputs of homeotic genes (*Hox*), abdominal A (*abdA*) and abdominal B (*abdB*). They specify glial fate by downregulating the levels of *CycE* in abdominal segments. On the contrary, the NGB6-4T represents a ground state which doesn't require input from any homeotic genes (Berger et al. 2005; Crews 2019).

After this overview of glial cell development, the subsequent section provides a brief survey of different glial cell types and their respective subtypes which have been documented in *Drosophila*.

4 *Drosophila* Glial Subtypes and Their Morphology

Intriguingly, during the course of evolution, the neurons to glial cell distribution within the CNS has expanded largely in favor of glia, reflecting its underlying functional significance. Glial cells contribute to about ~2% in the leech, ~15% in the fly, ~50% in mouse, and ~90% in the human central nervous system (Oberheim et al. 2006; Kremer et al. 2017). This progressive expansion of the glial population proportionally with the increasing complexity of the nervous system highlights its diversifying role(s) in regulating the homeostasis of the nervous system. In order to

establish a thorough understanding, there is a need for comprehensive investigation of glia cell types, structural anatomy, and their heterogeneous functions in the nervous system. However, such detailed analysis in the mammalian system is a strenuous task due to several limitations, and *Drosophila* offered a relatively efficient system for glial research. Besides offering a panoply of genetic-molecular tools and ease of culturing, *Drosophila* displays a plethora of glial cell types that are closely associated with the neurons. Intriguingly, growing work and meticulous examination of fly glia types strongly suggest that they exhibit substantial morphological as well as functional homology with the glial counterparts present in the mammalian system (Pfeiffer et al. 2010; Losada-Perez 2018). Therefore, performing exhaustive structural and functional analysis of *Drosophila* glial types can generate further insights into mammalian types.

Compared to several invertebrate models, *Drosophila* is a relatively complex organism and develops a complicated nervous system in a brief period of time, making fly as an impeccable model system to draw several parallelisms with the mammalian system (Crews 2019). The generalized architecture of the *Drosophila* CNS can be represented as two histologically distinct regions, the cortex that harbors the neuronal cell bodies and the core neuropils that sequester the synaptic connections (Meinertzhagen and Hanson 1993; Freeman 2015). Peripheral nerves which serve as the conduit between the CNS, the sensory organs, and musculature are additional constituents of the nervous system. Glial cells show a close association with all these anatomical structures essential for modulating neural circuit assembly, degeneration, and plasticity.

Extensive work on *Drosophila* has led to the identification of four major glial types based on their structure, topology, and expression of specific molecular markers (Edwards and Meinertzhagen 2010; Hartenstein 2011; Altenhein 2015). Of these, the midline glia represent a distinguished class of glial cells that appear early in the development and are specifically seen in the embryonic CNS. Another class of glia masks the entire CNS with the help of their sheathlike processes mimicking a canonical blood-brain barrier, known as the surface glia. Underneath the surface glia lay the cell body-associated glia or the cortex glia which encapsulate neuroblast cells and its daughter neuronal cell bodies packing them as discrete bunches in the cortical region of the CNS. As we approach toward the heart of the CNS, i.e., the neuropil, a diversified class of neuropil-associated glia can be observed, which are generally concentrated at the cortex and the neuropil interface. For better understanding, the respective arrangement of *Drosophila* glial types/subtypes and their localization within the CNS has been represented diagrammatically in Fig. 26.2.

A brief overview of different *Drosophila* glial type/subtype has been provided below:

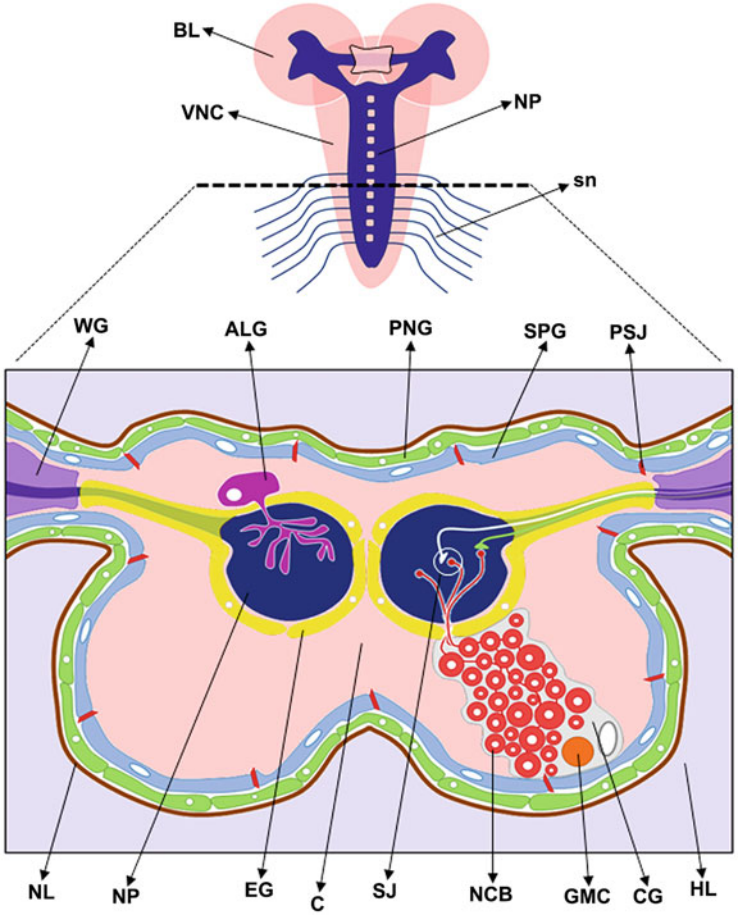

Fig. 26.2 Schematic representation of larval *Drosophila* central nervous system. The larval brain is a tri-lobular structure comprising of two spherical brain lobes (BL) and a longitudinally extending ventral nerve cord (VNC). All neuronal cell bodies project their axons penetrating deep into the neuropil (NP), harboring extensive network of synaptic junctions (SJ). Several segmental neurons (sn) connect the CNS with the peripheral nervous system (PNS). The dotted red line drawn across the VNC represents the site of cross section. The transverse section represents the morphology and topology of the various glial cell types present in the CNS. The entire CNS can be differentiated into two histologically different zones – the cortex (C) and the neuropil (NP). The entire brain is metabolically insulated from the hemolymph (HL) with help of a glial blood-brain barrier composed of two distinct layers, an outer perineurial glia (PNG) and an inner subperineurial glia (SPG). The PNG secretes a tough extracellular matrix, neural lamella (NL), providing a definite shape to the CNS, whereas SPG is tightly packed with the help of pleated septate junctions (PSJ) restricting paracellular diffusion. The neuronal cell bodies (NCB) and ganglionic mother cells (GMC) lay in the cortex which are engulfed by the cortex glia (CG). The neuropil is covered entirely with ensheathing glia (EG) and is densely infiltrated by filopodial extensions of astrocyte-like glia (ALG). Wrapping glia (WG) ensheathes long motor and sensory axons of the PNS. White circles within each cell represent the shape and location of the nuclei

4.1 Midline Glia

The midline glia are considered as a unique class of glia due to its mesodermal origin and unambiguous occurrence in the embryonic CNS (Jacobs 2000). Interestingly, these groups of cells have a distinct pattern of gene expression compared to those observed in other glial cell types. The most striking feature of midline glia is that despite their glial identity, they fail to express the conventional glial markers, the *gcm* and *repo*, opening an avenue to explore other molecular pathways that provide them their glial fate (Crews 2019). Due to their stereotypical presence in the embryonic CNS, they most likely facilitate some cardinal events that occur during the early developmental phase of the organism.

The midline glia predominantly function in axonal guidance during embryonic CNS development (Freeman 2015). Some other major functions include providing trophic support to commissural axons, influencing the migration and formation of neighboring cell types, wrapping of the commissural axons that cross the CNS during embryonic development, and locomotor function (Crews 2010; Freeman 2015; Ismail et al. 2019).

Taken together, *Drosophila* midline glia are partially regarded as reminiscent mammalian radial glia as both function in axon guidance in early neurogenesis, subsequently vanishing in the mature or adult nervous system (Freeman and Doherty 2006; Zuchero and Barres 2015). In the line of above, it is quite evident that with appropriate molecular and genetic tools, *Drosophila* midline glia can be modeled to study their role in some fundamental aspects of neurogenesis.

4.2 Surface Glia

The nervous system is the most sophisticated and delicate system found in the organisms across the entire animal kingdom. Organisms endowed with a complex nervous system are generally well-fortified and are metabolically isolated from the rest of the body due to the presence of a robust blood-brain barrier (Abbott 2005; Harilal et al. 2020). An active blood-brain barrier composed of surface glia also provides chemical and mechanical protection from the hemolymph in the case of *Drosophila* (Abbott et al. 2006; Tietz and Engelhardt 2015). There are two distinct surface glia, namely, the perineurial glia (the outer layer) and subperineurial glia (the inner layer), that contributes to the fly blood-brain barrier (Yildirim et al. 2019). The subperineurial layer develops first at the time of late embryogenesis followed by the formation of perineurial layer during larval development (Awasaki et al. 2008; Hartenstein 2011). Both the cell layers form an efficient mechanical boundary restricting the chemical communication across the barrier permitting only selective influx of molecules and organic compounds (Featherstone 2011; Hindle and Bainton 2014). The number and size are inversely correlated to both the layers, perineurial glia being small but numerous while subperineurial glia being large flat cells but fewer. Besides the differences in the physical parameters, both the cell types display

significant differences in their framework and architecture, promoting proper division of labor among themselves (Kremer et al. 2017).

The surface glia cover the entire surface of the CNS and PNS (Ito et al. 1995; Awasaki et al. 2008; Kremer et al. 2017). They form a metabolic barrier which separates the *Drosophila* hemolymph from the nervous system (Yildirim et al. 2019). The outermost layer of the blood-brain barrier is made by the cells of perineurial glia, which are cushioned between the dense neural lamella contributed by itself on the side of hemolymph and subperineurial glial layer on the side of the nervous system (Volkenhoff et al. 2015; Yildirim et al. 2019). Beneath the perineurial glia lies the layer of subperineurial glial cells, which contribute to the formation of blood-brain barrier by insulating intercellular septate junctions; therefore, paracellular diffusion is restricted, and a control mechanism determines the movement of all hydrophilic molecules in or out of the nervous system (Baumgartner et al. 1996; Schwabe et al. 2005; DeSalvo et al. 2011). Therefore, both of these glial cell types play role in the generation and maintenance of the blood-brain barrier, with subperineurial glia working for the paracellular barrier and perineurial glia providing the physical support for its maintenance (Kremer et al. 2017). The transcriptome analysis of the subperineurial glia and perineurial glia reveals that these glial cells possess a variety of evolutionarily conserved properties/structures which aid in the maintenance of the nutrient/ion homeostasis and drug efflux (DeSalvo et al. 2014). Some additional functions of these two glial types have been further elaborated below.

Perineurial Glia

The perineurial glia form the outermost layer of the blood-brain barrier covering the entire nervous system. The layer is sandwiched between hemolymph on one side and by a layer of tightly packed subperineurial glia on the other. To reinforce the structure, perineurial glia vigorously secrete and deposit an extracellular matrix called neural lamella preventing its direct exposure to the hemolymph (Kim et al. 2014). Due to its characteristic alignment between neural lamella and subperineurial glia, the perineurial glia hardly contact any neuron except those found in the developing eye (Sasse et al. 2015). Perineurial glial cells originate during embryogenesis but undergo functional differentiation at the time of early larval development, retaining mitotic activity throughout the larval stages (Altenhein et al. 2016). This property ensures that perineurial glia are able to compensate for the increasing demand for masking the dramatically enlarged surface area of the mature nervous system (Subramanian et al. 2017).

Interestingly, besides proliferative property, perineurial glia exhibit a dynamic morphology throughout the lifetime of the organism, transforming from a spindle shape with fine filopodial projections during the larval stage to a dorsoventrally expanded form in adults, forming an overall loose array of cells with their lamellar projections forming a meshwork (Awasaki et al. 2008; Stork et al. 2008; Yildirim et al. 2019). Although the exact function of this layer is still elusive, however, since the perineurial glia are an indispensable component of the blood-brain barrier, it is selectively permeable in nature and allows selective molecular transport across

it. The selectivity of this layer can be attributed to the expression of several transport proteins crucial for shuttling nutrients and ions (Brankatschk et al. 2014; Limmer et al. 2014; Volkenhoff et al. 2015). A little is known about the interacting partners that aid in precise communication between the perineurial glia and neural lemma, and identification of such glial regulators is still under investigation (Hunter et al. 2020).

In addition to providing nutrients, the perineurial glial cells also express heparan sulfate proteoglycan, Dally-like protein (Dlp), which itself regulates neuroblast proliferation (Kanai et al. 2018). During development, perineurial glial cells also play a significant role during the process of brain compaction and growth. The CNS tends to compact during the embryonic stages and selectively grows in the course of larval development. The size of the neural lamella is also then modified according to the growing nervous system. Since perineurial glial cells contribute to the formation of this neural lamella, it has been shown that they control the stiffness of the same via secreting proteases, and any imbalance in their activity leads to abnormal shape of the nervous system (Meyer et al. 2014).

Subperineurial Glia

As already mentioned, a layer of subperineurial glia forms the inner component of the blood-brain barrier lying directly beneath the perineurial glial layer. It functions as an additional supportive layer and defends the nervous system efficiently. The subperineurial glial cells are one of the glial types that emerge during embryogenesis and persist throughout the lifetime of the organism with minimal transformation in shape and structure (Hartenstein 2011; Kremer et al. 2017). The subperineurial glial layer possesses characteristically large flattened cells and nuclei enveloping the entire larval brain. The subperineurial glial cells are well-interdigitated cells with pleated septate junction sealing the system resembling a tightly tiled epithelium (Stork et al. 2008; Mayer et al. 2009). A tight epithelium-like arrangement overcomes the structural limitation which was persistent in the mesh-like architecture of the perineurial glia.

As mentioned earlier, the subperineurial glia exist in fewer number; for instance, only about ~20 subperineurial glial cells mask each of the larval brain hemispheres (Pereanu et al. 2005; Stork et al. 2008). Since there is a gradual increment in the surface area of the brain, the number of subperineurial glia must also increase. Intriguingly, subperineurial glia undergo phenomenal endoreplication and endomitosis, to enhance their overall size in order to fulfill the necessity, rather than proliferating at a fast rate (Unhavaithaya and Orr-Weaver 2012). The number of subperineurial glial cells rises to ~300 in the adult brain and ventral nerve cord, contributing up to ~2% of the total glial population (Kremer et al. 2017). Compared to the perineurial glia, the subperineurial glial cells manifest an intimate connection with the most superficial layer of neuron cell bodies housed in the cortical region (Freeman 2015). Besides septate junctions, other junctions such as spot adherence junction and gap junctions establish an intracellular link across the individual subperineurial glial cells and between subperineurial glia and the cortex glia lamella located beneath its surface (Pereanu et al. 2005). This interaction between the

subperineurial glia and cortex glia establishes a microenvironment that regulates the proliferation of neuroblast cells (Spéder and Brand 2014, 2018).

In addition to the above, subperineurial glial cells are also involved in several other functions. The subperineurial glial cells are known to spread out processes that interact with the synaptic contacts in the motor neurons of the muscles, at the neuromuscular junction. There, they accomplish various roles such as (i) recycling of the neurotransmitters (Rival et al. 2004; Danjo et al. 2011; Chaturvedi et al. 2014), (ii) shaping of the growing presynaptic morphology by consuming axonal/synaptic debris shed in course of development (Fuentes-Medel et al. 2009; Freeman 2015), (iii) secretion of the molecules such as transforming growth factor, (TGF)-β, that influence retrograde signaling during synapse formation (Fuentes-Medel et al. 2012), and (iv) secretion of glycoproteins, Wnts, that control synaptic physiology and clustering of the glutamate receptor at the neuromuscular junction (Kerr et al. 2014; Freeman 2015).

Also, the subperineurial glial cells form lipid droplets in hypoxic and oxidative stress conditions, and these droplets eventually aid in the protection of neuronal stem cells from oxidative damage (Bailey et al. 2015). The subperineurial glial cells are further considered as controllers that monitor the reactivation of quiescent neuronal stem cells in response to nutritional cues (Spéder and Brand 2014).

4.3 Cortex Glia

The cortex glia also referred to as cell body-associated glia occupy the rind of the brain filling most of the cortical region. The cortical zone of a CNS is known to sequester the neuronal cell bodies. Since neurons are sealed away from the hemolymph, their requirements for raw materials are fulfilled by the cortex glia, which functions as a mediator between the neurons and the subperineurial glia (Stork et al. 2012). For efficient channelization of the nutrients, the cortex glia extend its lamellar projections and engulf a large population of neurons (~55 up to 100 neurons), ganglion mother cell, and neuronal cell extensions (Pereanu et al. 2005; Coutinho-Budd et al. 2017; Spéder and Brand 2018). The cortex glia and the neuronal cell bodies are held together with the help of adherence and septate junctions, maintaining the structural integrity of the entity (Hartenstein 2011). Thus, in a mature brain, cortex glia form an integrated complex with the neuronal cell bodies generating a honeycomb-like structure, which is often referred to as "trophospongium," which presumably supports the neuronal cell bodies and the proximal region of the neurites before they actually reach and innervate the neuropil (Freeman 2015). Since the cortex glia extend along the radii of the cortical region, its apical processes interact with the surface glia and neuroblast cells, whereas its basal processes partially contribute to the formation of a thick glial lamella, also referred to as "neuropil glial sheath" separating the cortex from neuropile (Spindler et al. 2009).

The cortex glial cell covers individual neuronal cell body and extends trophic support to neurons (Ito et al. 1995; Stork et al. 2012; Kremer et al. 2017). Cells of the cortex glia contribute in providing metabolic support to neurons through the

expression of a lactate/pyruvate transporter named Chaski, which is important for suitable synaptic transmission (Delgado et al. 2018). In addition to the metabolic and trophic support, these glial cells are also known to contribute to the regulation of glia-neuron communication. Involvement of cortex glia in Ca^{2+}/calmodulin-dependent signaling pathway aids in regulating the glia-neuron communication (Melom and Littleton 2013). Interestingly, severe disruption in the glial Ca^{2+} signaling pathway elicits seizures in *Drosophila*, suggesting a probable role of glial cells in epileptic pathology (Melom and Littleton 2013).

The cortex glia also associate with the other glial types for administration of additional functions. The cortex glia cells associate with subperineurial glial cell layer of the blood-brain barrier to act as channels for the proficient transmission of nutrients from the hemolymph to the neuronal cell bodies. Gas exchange is also enabled via these cells as they tend to make substantial contact with the fly vasculature (Pereanu et al. 2005; Freeman 2015). Remarkably, studies suggest a direct association between the cortex glia and astrocyte-like glia (Farca Luna et al. 2017). Inhibition of Amyloid precursor protein homolog, Appl, in the cortex glial cells has been found to elicit changes in the astrocyte-like glia, and this suggests a direct link between the two. Here it is important to note that Appl is predominantly required in the cortex glial cells for the regulation of sleep/awake cycle, and its inhibition in cortex glia causes higher sleep amounts in *Drosophila*. This correlates with the changes observed in the astrocyte-like glial cells in terms of glutamine synthetase expression and Innexin 2 (prime constituent of the glial gap junctions at the cortex and astrocyte-like glial cell interface), suggesting a potential link between these two glial types (Farca Luna et al. 2017).

Despite their remarkable morphology and critical function of nourishing the neurons, cortical glia are one of the most understudied type of glia and would be an interesting glial cell type for further exploration.

4.4 Neuropil-Associated Glia

As we progress toward the deeper regions of the brain, we reach to the core of the nervous system, the neuropil. The neuropil largely accommodates all the dendrites, axons, and synaptic junctions (Freeman 2015). The neuropil is surrounded by two differentially localized neuropil-associated glial cell types – the astrocyte-like glia and the ensheathing glia. Both the cell types originate from the embryonic longitudinal glioblast cells (Omoto et al. 2015). Mitotic division of this cell during embryogenesis yields six astrocyte-like glial cells, two lining the neuropil and one lining both the neuropil and the nerve roots, and the latter three are referred to as the ensheathing glia (Peco et al. 2016; Stacey et al. 2010; Stork et al. 2014). Gradually, the larval astrocyte-like glial cells and the ensheathing glial cells undergo programmed cell death during metamorphosis and get replaced by newly formed astrocyte-like glial cells and ensheathing glial cells (Omoto et al. 2015). Since the primary/larval neuropil-associated glial cells fail to contribute to the adult brain, it gives rise to the need for another round of mitosis. Therefore, the adult population of

neuropil glial cells arises from the division of type 2 neuroblast cells that participate as secondary precursor cells (Omoto et al. 2015). The resulting progeny cells are flattened, firmly aligned along the boundaries of the neuropil, and heavily concentrated near the central neuropil complex (Hartenstein 2011). The cells are distributed between the neuropil compartments, defining the neuropil and cortical interface. Despite both the cell types (astrocyte-like glial cells and ensheathing glial cells) sharing common progenitor cells, they have drastically different structural as well as functional properties as summarized below.

Astrocyte-Like Glia

The astrocyte-like glial cells possess one of the most intricate and complex morphologies among of all them (Freeman 2015). Interestingly, this is one of the most studied glial cell types and is found in abundance ranging up to ~ 4,500 cells in an adult CNS (Kremer et al. 2017). The corresponding cells manifest a large rounded cell body residing between the sheaths laid by the ensheathing glia over the neuropil (Omoto et al. 2015; Stork et al. 2014). Due to resemblance in nomenclature shared with the vertebrate type, the *Drosophila* astrocyte-like glia are also referred to as "reticular glia" by several authors (Hartenstein 2011). The astrocyte-like glial cells are highly polarized and display a variable number of dense and delicate branched processes that infiltrate the neuropil, covering a vast majority of synaptic gaps as they mature (Muthukumar et al. 2014; MacNamee et al. 2016; Omoto et al. 2015; Peco et al. 2016). This intricate morphogenesis in the young astrocyte-like glia is attributed to astrocyte-neuron FGF (Fibroblast Growth Factor) signaling cascade (Stork et al. 2014). Comprehensive analysis of astrocyte-like glia revealed that the cell body and initial branches are microtubule-rich, whereas the fine processes are actin-rich, vindicating their polarized nature (Stork et al. 2014). The astrocyte-like glial cells show notable interaction as well as tiling effect among themselves to ensure effective coverage of the synapse-rich neuropil (Stork et al. 2014). Upon their ablation, they exhibit an astonishing property of expanding into areas deprived of astrocyte-like glial cells (Stork et al. 2014). Intriguingly, with recent advancements in better visualization aids, it has been demonstrated that a single astrocyte can innervate multiple neuropils at the same time (Kremer et al. 2017).

Drosophila astrocyte-like glia show significant functional similarities to their mammalian counterparts, the protoplasmic astrocytes (Stork et al. 2014; Freeman 2015). The function of astrocyte-like glia appears to be dependent upon the close relationship between synapses (Freeman 2015). The astrocyte-like glial cells play an important role in formation of appropriate synapse connections in the fly brain (Muthukumar et al. 2014). In addition, they host receptors for neurotransmitter release and homeostasis, such as EAAT1 (excitatory amino acid transporter 1) for glutamate (Rival et al. 2004) and GAT (GABA transporter) for GABA (gamma-aminobutyric acid) (Stork et al. 2014). This glial type is vital for neurotransmitter clearance from the synaptic area, as loss of this property makes negative impact on survival and behavior of *Drosophila* (Freeman 2015). Interestingly, depletion of EAAT1 in adult *Drosophila* astrocyte-like glia causes neurodegeneration and shortened life span (Rival et al. 2004). Further, it has also been reported that the astrocytic

GAT activity is found to be critical for normal motor function and viability in *Drosophila*, as its partial loss leads to distorted locomotion and GAT null mutants die at late embryonic stage. Astrocyte-like glia facilitated GABA clearance through GAT is proposed to be the potential responsible factor for the above (Stork et al. 2014).

Other than neurotransmitter homeostasis, astrocyte-like glial cells also aid in neurite pruning and subsequent clearance of pruned neurite debris (Kremer et al. 2017). To facilitate this clearance, the astrocyte-like glia converts during the initiation of metamorphosis to a phagocytic cell which engulfs the pruned debris within the neuropil (Awasaki et al. 2011; Hakim et al. 2014; Tasdemir-Yilmaz and Freeman 2014).

The major challenge in investigating the in-depths of astrocyte-like glia was dissecting out the molecular mechanism that underlies their complex architecture, synaptic plasticity, and astrocyte-neuron communication and has been of great interest to several developmental and neurobiologists (Mederos et al. 2018).

Ensheathing Glia

The neuropil-associated glia also differentiate into the ensheathing glia, which exhibit a relatively basic phenotype as compared to that of astrocyte-like glia. The ensheathing glial cells dominantly associate with the axonal tracts. These characteristically flattened cells are devoid of long and prominent surface processes that might penetrate deep into the neuropil compartment (Doherty et al. 2009). Contrary to astrocyte-like glia, ensheathing glia sends minute processes into the nerve root and some major axon bundles that innervate the neuropil (Pereanu et al. 2007). The cells occasionally extend flattened processes along the circumference of the neuropil, acquiring a sheet or lamella-like morphology, which eventually bends or folds up to form a tubelike structure covering a wide array of shapes and forms (Hartenstein 2011). This distinct arrangement of ensheathing glia is attained due to the close association of both the ensheathing glia and cortex glia with the ganglionic mother cells and its newly born progeny cells (Dumstrei et al. 2003). As the neuroblasts divide and produce daughter cells, they spread axons toward the neuropil. Ensheathing glial cells surround the fiber tracts when they enter the neuropil and potentially establish early boundaries that delineate brain lobes and separate the neuropil from the cell cortex (Freeman 2015). Recently, after meticulous analysis of its localization and occurrence, ensheathing glia are specifically used to denote all the glial cells lying within the CNS that wraps the neuropil and axons or axon fascicles that connect the neuropil to its periphery (Yildirim et al. 2019). Taken together, ensheathing glia is confined to the CNS and around those neuronal projections that lie within the territory of CNS.

Ensheathing glia have been reported to be essential for morphogenesis of CNS (Spindler et al. 2009; Yildirim et al. 2019). A critical role of neuropil and cortex glial cells has been suggested during axon formation and growth in *Drosophila* larvae, and removal of neuropil-associated glial cells comprising ensheathing glia generates deficiencies in the axon tract formation and subsequent alterations in their trajectories (Spindler et al. 2009).

In addition to regulating CNS morphogenesis, it has also been demonstrated that ensheathing glial cells potentially remove the axonal debris from the neuropil by functioning as phagocytic regulators of axonal debris, utilizing Draper engulfment receptor (Doherty et al. 2009). Their role as phagocytic entities was established after the axons of olfactory receptor neurons (ORN) of the maxillary palp were damaged in a fly, in which the Draper engulfment receptor is expressed by ensheathing glia. Moreover, expression of Draper was also downregulated in such condition, and subsequent clearance of the axonal debris was completely blocked (Doherty et al. 2009). This evidently demonstrates the involvement of ensheathing glia in phagocytosis of the axonal debris. It has also been reported that post injury, the ensheathing glial cells utilize a positive autoregulatory loop mechanism to regulate their responsive state to injury debris engulfment (Doherty et al. 2014). This autoregulatory loop mechanism involves a signaling cascade mediating the engulfment receptor Draper, molecules of Src kinase family, and additional molecules of glial membrane recruitment, debris internalization, and acidification. Through this positive autoregulation, Draper also enhances its own *draper* gene expression via signaling to the nucleus (Doherty et al. 2014; Freeman 2015).

The ensheathing glial cells are also known to modulate the synaptic plasticity of the local circuits via an injury response cascade. In this, the damaged sensory axons recruit glia, which further signal to upregulate the activity of the central neurons (Kazama et al. 2011). Post axotomy, the olfactory receptor neuron synapses onto the projection neurons in the antennal lobe of the adult fly brain. The repression in the generation of projection neuron plasticity was ensued after the blockage of endocytosis in ensheathing glial cells. This signifies that ensheathing glia perhaps regulate plasticity of the connections of the projection neurons in the olfactory circuit cascade, possibly to enhance the activity in the leftover neurons for compensation of the missing sensory input (Kazama et al. 2011).

In addition to the above, the ensheathing glial cells have also been reported to regulate the function and processing of the neural network. This involves a sulfite oxidase encoding *shopper* gene, which specifically required in ensheathing glia to control head bending and peristalsis in *Drosophila* larvae, via glutamate-glutamine cycle. Mutation in *shopper* causes increased level of sulfite and disturbs glutamate homeostasis. This subsequently influences the neuronal network function and alters the head bending frequency (Otto et al. 2018). Interestingly, a mutation in *shopper* homolog in humans also causes comparable neurological symptoms such as seizures (Otto et al. 2018). Such similarities between *Drosophila* and humans open new possibilities for in-depth tracing of the mechanistic details of the functioning of ensheathing glial cells and their potential involvement in development of neurological disorders.

Interestingly, another glial subtype, the wrapping glia exhibits notable resemblance with the ensheathing glia. However, the wrapping glial cells appear to be engaged only with the axonal structures of the PNS. A brief overview of wrapping glia has been provided below.

Wrapping Glia

Wrapping glia share substantial functional and structural homology with ensheathing glia (Kremer et al. 2017). This type of glia gained attention during the study of the developing optic and abdominal nerves. Wrapping glia are exclusive to the PNS and enclose both the sensory and motor neurons, flanked by CNS and sensory organs. Largely, they envelop the long axonal tracts, and about three to four wrapping glial cells are required to cover a 3 mm long nerve fiber (von Hilchen et al. 2013; Matzat et al. 2015). As the organism matures, the wrapping glia differentiate with an increasing concealing efficiency (Matzat et al. 2015). In addition to wrapping the sensory and motor axons, wrapping glia also hold the capacity to affect the differentiation and proliferation of the subperineurial glia and perineurial glia, respectively. While doing so, the wrapping glial cells act as a center point for the development of the different glial layers of the peripheral nerves (Matzat et al. 2015).

It is increasingly evident now that each of the glial type/subtype found in *Drosophila* manifests a distinct morphology and function. Since glial cells are the key structural elements of the nervous system, hence, studying the repercussions of any structural anomaly or deformities in glial cells is of prime interest before altering their metabolic role. Performing such experiments in vivo in the mammalian system is a tedious task; however, a tiny invertebrate *Drosophila* offers an opportunity to address and explore several questions such as the manifestation of structural ablations, the physical role of glial cells during neurogenesis, synapse formation and pruning, blood-brain barrier formation, neuroprotection, etc. In addition, these particulars of the *Drosophila* glial system would also help in elucidating how any dysfunction in their structural or functional aspects could contribute to abnormal conditions or disease etiology. Taken together, *Drosophila* can prove to be a promising system to model several conditions and to derive answers for some fundamental events in glial cells which can be extrapolated to the mammalian counterparts. In the line of the above, the subsequent texts offer a brief comparison between *Drosophila* and mammalian glia at various levels.

5 Anatomical Comparison of Mammalian and *Drosophila* Glial Subtypes

Based on the morphological signature(s), four major types of glia have been categorized in mammals, as enlisted in Table 26.3. Astrocytes, the star-shaped cells, are among the most abundant cell type in the brain whose conventional function is to provide structural support to neurons in CNS. Oligodendrocytes are the ensheathing glial cell types which form an envelope around axons of CNS referred to as myelin sheath and help in saltatory conduction among neurons. Microglia are the cells with the hematopoietic origin and are the immune cells of CNS. Lastly, Schwann cells ensheath the peripheral nerves in the PNS, and they can be both myelinating and non-myelinating (Nayak et al. 2014).

Table 26.3 Anatomical and morphological comparison between vertebrate and *Drosophila* glial cell types

Vertebrate glial cell types	Spatial distribution	Primary cellular function	*Drosophila* glial counterpart
Astrocytes	Embedded in the cellular cortex of CNS, CNS surface	Provide structural support to neurons, synapse modulation	Cortex glia along with subset of surface glia
Oligodendrocytes	Wrap around axons of CNS	Ensheath neurons, provide trophic support, myelination	Neuropil glia
Microglia	All over CNS	Act as macrophages of CNS, perform immune function	Cortex, neuropil, surface glia
Schwann cells	Wrap axons of peripheral nerves	Wrap around peripheral nerves to provide support, myelination	Peripheral glia

In *Drosophila*, the main categories of the CNS glia are primarily the cortex, neuropil, surface, and peripheral glia. They exhibit anatomical and functional similarities with their mammalian counterparts as enlisted in Table 26.3. As already mentioned above, cortex glia also known as cell body-associated glia are structurally very similar to the astrocytes and are embedded deep in the cellular cortex establishing close physical contact with neurons. They extend their cellular projections around cell bodies of neurons making a honeycomb structure that enters deeply between spaces of neuronal cell bodies (Pereanu et al. 2005). Intriguingly, they also make surface contacts with oxygen supplying tracheal elements and with the blood-brain barrier (Pereanu et al. 2005; Ito et al. 1995). Similarly, the mammalian astrocytes also act as cellular channels that are responsible for supplying gases and nutrients to target neurons of CNS. The neuropil glia appear to function like oligodendrocyte cells of the mammalian system as they extend their membranous sheath around the targeted axon/axon bundles helping in the formation of properly fasciculated nerves (Ito et al. 1995; Klämbt et al. 1991). They form an insulating environment around the axons which protects them in an environment conducive to neuronal firing. Moreover, they also promote neuronal survival through trophic support mechanisms (Booth et al. 2000). Interestingly, there is no glial cell-type in *Drosophila* which specifically performs immune functions; instead, all glial cells appear to have the capability to execute functions like of immune cells, such as engulfment of neuronal corpuses during development (Sonnenfeld and Jacobs 1995; Freeman et al. 2003). The peripheral glial cells, like Schwann cells, ensheath and maintain peripheral nerves having both sensory and motor axons (Auld et al. 1995; Leiserson et al. 2000).

The ectoderm-derived surface glia form a blood-brain barrier in *Drosophila* CNS, consisting of a sheath of a flattened layer of surface glial cells that helps in separating the neuronal tissues from the rest of surrounding tissues and hemolymph (Edwards et al. 1993; Ito et al. 1995). A pleated septate junction is formed between surface glia and in between the surface and cortex glia (Pereanu et al. 2005). These septate

junctions are composed of neurexin IV, contactin, and neuroglian (Bhat et al. 2001). On the other hand, the mammalian blood-brain barrier is derived from the specialized cerebral endothelial cells forming tight junctions and acts as a barrier. In addition, these tight junctions get surrounded by the basement layer secreted by mesoderm-derived pericytes. The blood vessels are almost surrounded by astrocyte end feet and considered as major sites that uptake nutrients by capillaries and direct them to targeted neurons by astrocytes (Nayak et al. 2014). Here it is important to note that despite this morphological dissimilarity between the mammalian and *Drosophila* blood-brain barrier, they seem to function in an extremely analogous manner, each capable of defending the uptake of passage ions and small molecules (Juang and Carlson 1994).

Taken together, it is increasingly clear now that *Drosophila* glial system is undeniably similar to the mammalian glial system. They share a comparable pattern of development, function, and morphology and perhaps have similarity in the molecular criteria as summarized in Table 26.3. Such undeniable similarity postulates *Drosophila* as a great model organism that could be utilized not only to unfold the extensive genetic networks involved in establishing glial biology but also to decipher their involvement in pathogenesis and progression of devastating human neurodegenerative disorders. The subsequent sections of this chapter provide a brief overview of the contribution of the *Drosophila* in revealing the association of glial cells in human neurodegenerative diseases.

6 The Role of Glia in Neurodegeneration

As discussed earlier, it is well-established now that the glial cells play an indispensable role in maintaining the neuronal shape, nutrition, and homeostasis. Also, they ensheath the neurons and establish electrical isolation, and they maintain axon guidance and help in establishing the blood-brain barrier (Kretzschmar and Pflugfelder 2002; Chotard and Salecker 2007). Hence, due to the essential role (s) of glial system in maintaining the integrity and functioning of the neuronal cells, investigating the significance of the glial cells in development of brain disorders has emerged as a prime area of neurobiology.

Neurodegenerative disorders are a group of age-onset disorders of the nervous system, comprising of tauopathies, polyglutamine diseases, atrophies, ataxias, etc. Due to the complexities and limitations attached with human genetics and higher mammalian models, it is relatively difficult to investigate the underlying mechanisms of these complex disorders. Therefore, relatively simpler organisms such as *C. elegans*, *Drosophila*, zebrafish, etc. have emerged as valuable tool to investigate the in-depths of these disorders. Specially, *Drosophila* serves as an excellent model to study the human neurodegenerative disorders due to several advantages such as the presence of at least one homologous gene for almost 75% of human disease-causing genes and the availability of a wide range of tools for genetic manipulation (Hirth 2010). Further, similar to the mammalian brain, the distinct regions of adult *Drosophila* brain are capable of carrying out complex

behaviors such as learning and memory; and such abilities further qualify this organism as a prime model for neurobiology research. Here, it is also worth noting that *Drosophila* provides a multitude of mutant lines which serve as neurodegenerative disease models, as well as several transgenic lines of human neurodegenerative diseases which beautifully recapitulate all the hallmarks of the mammalian neurodegenerative disorders such as memory loss, locomotory defects, cognitive impairments, accumulation of proteinaceous aggregates, cognitive impairments, and neurodegeneration (Lessing and Bonini 2009; Lu and Vogel 2009; Mohylyak and Chernyk 2017).

Interestingly, in addition to the CNS, the fly's visual system also serves as an excellent system to study the glia-neuron interactions. The fly eyes are a complex of neuronal as well as nonneuronal cells; therefore, there is an abundance of glial cells in their visual system. The glial cells in the visual system carry out all the processes similar to the brain glia including metabolism and sanitation of the eye neuronal cells and recycling of the neurotransmitters released from the photoreceptor neurons (Augustin et al. 2007; Borycz et al. 2012; Borycz et al. 2002; Chaturvedi et al. 2014; Pantazis et al. 2008; Rahman et al. 2012; Romero-Calderón et al. 2008). This intimate physiological association between the eye-specific neurons and glia makes *Drosophila* eyes a model tissue to investigate the association of glial cells in neurodegeneration.

The first *Drosophila* neurodegenerative mutant to be discovered was *drd* (*drop dead*) (Hotta and Benzer 1972). The mutants such as *drd*, and others like *swiss cheese* [*sws*; a fly ortholog of mammalian neuropathy target esterase (NTE)] and *repo* (reversed polarity), develop neurodegeneration due to pathological changes in glial cells (Mohylyak and Chernyk 2017). The first study to hint the association between glial cells and neurodegeneration was carried out in a *repo* (a glia-specific protein) mutant line. These *repo* mutants showed undifferentiated laminar glial cells and were ultimately eliminated by programmed cell death. Interestingly, the neurons in the lamina and retina also displayed a similar degeneration pattern, and this suggested the significance of glial cells in neurodegeneration, for the first time in the fly system (Xiong and Montell 1995). Here, it is also worth noting that majority of the mutant lines with neurodegeneration phenotypes exhibited degeneration in both neurons and glial cells. In addition, behavioral changes and locomotor defects were also noted in some of the mutant lines, including the *sws* mutants, due to changes in only the glial cells (Kretzschmar et al. 1997). The major glial changes contributing in developing such behavioral defects could be the loss of insulation and modulation of the neurons, which in turn causes lack of coordination between the neuronal responses (Dutta et al. 2016).

Most of the studies performed to investigate the relationship between glial degeneration and neurodegeneration were focused on the myelination process. Studies on *sws* mutants suggested that these flies are defective in the wrapping/ensheathing of the neuronal exons, which is the most likely explanation of the neurodegenerative phenotype in these flies (Kretzschmar et al. 1997; Dutta et al. 2016). Though some studies in mice model have noted that fully myelinated neurons could also degenerate (Griffiths et al. 1998), it appears that degeneration of axons

may not be related to the myelination process in all the cases. In view of the fact that the axons in the fly system are not fully myelinated, hence, the fly system provides an opportunity to unravel the novel glial proteins involved in neurodegeneration. This again makes the *Drosophila* a highly relevant system to dissect the role of the glia in the pathogenesis of the neurodegenerative disorders, not limited to myelination alone (Mohylyak and Chernyk 2017).

6.1 Mechanism of Glial Pathogenesis in Neurodegeneration: Answers from Fly Models

A phenomenon known as reactive gliosis, involving activated microglia, is commonly found in patients suffering from various neurodegenerative diseases such as Alzheimer's disease, Creutzfeldt-Jakob disease, and Huntington's disease (Heneka et al. 2010; Sarlus and Heneka 2017; Joers et al. 2017; Novellino et al. 2020). Activated microglia is a rapid immune response reaction that they develop on sensing pathological changes in the brain. It has been suggested that such inflammatory reaction or oxidative stress may trigger neurodegeneration mechanisms (Lull and Block 2010). Reactive gliosis brings about changes in the gene expression such that the processes required for utilization of dying neurons by glia are triggered. The *Drosophila* hemocytes are considered as the functional counterparts of mammalian microglia (Kretzschmar and Pflugfelder 2002; Mohylyak and Chernyk 2017). The following section provides an insight into the role of glia in the pathogenesis and protection of different neurodegenerative models in *Drosophila*.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS)/Lou Gehrig's disease is a neurodegenerative disorder associated with dominant mutations in Cu-Zn *superoxide dismutase* (SOD1) gene (Rosen et al. 1993). The pathogenesis and symptoms of the disease are preceded by astroglial degeneration (Rossi and Volterra 2009), both in humans and in transgenic animal models (McGeer and McGeer 2002; Rossi and Volterra 2009). As the disease progresses, the astrocytes become reactive and begin to release neurotoxic factors and facilitate microglial activation (Nagai et al. 2007; Rossi and Volterra 2009). The exact mechanism by which the microglial cells become activated in amyotrophic lateral sclerosis is yet to be determined; however, the inflammatory component remains as the major contributor.

Parkinson's Disease

Parkinson's disease is another neurodegenerative disorder which primarily affects the dopaminergic neurons in the substantia nigra. The characteristic features of Parkinson's disease include impaired locomotor such as rigidity and tremor. Interestingly, profound changes in the astrocytes (McGeer and McGeer 2008; Mena and Garcia de Yebenes 2008) and microglial activation (Kim et al. 2009; Theodore et al. 2008) have been noted in the substantia nigra during the later stages of the disease. Moreover, the presence of pathological Lewy bodies, α-synuclein inclusions that are

abundantly found in the neurons (Baba et al. 1998; Beyer and Ariza 2007), was also observed to some extent in astrocytes (Braak et al. 2007; Song et al. 2009; Wakabayashi et al. 2000). Interestingly, targeted expression of α-synuclein in glial cells leads to the formation of glial inclusions and locomotory impairments and causes autonomic dysfunction and ultimately induces the death of dopaminergic neurons in Parkinson's disease model of *Drosophila* (Feany and Bender 2000; Ordonez et al. 2018). Here, it is also important to note that α-synuclein inclusions in dopaminergic neurons increase substantially upon its co-expression in both the neurons and glia, as compared to its expression in neurons alone (Olsen and Feany 2019). This suggests an imperative role of glial α-synuclein in perpetuating pathogenic α-synuclein aggregation in dopaminergic neurons in a non-cell-autonomous manner (Olsen and Feany 2019). Thus, glial α-synuclein significantly enhances the pathological as well as the clinical hallmarks of the Parkinson's disease.

Alzheimer's Disease

Intriguingly, glial involvement in Alzheimer's disease, for the first time, was suggested by Alois Alzheimer himself. He demonstrated that glial cells were a part of the neuronal amyloid plaques in association with the neurofibrillary tangles (NFTs) (Alzheimer 1910). The amyloid plaques in the Alzheimer's disease brain have been found to be surrounded by the activated astrocytes (Nagele et al. 2004; Rodriguez et al. 2009). Oxidative stress in astrocytes is one of the major causes of neuronal deaths as demonstrated in the cell culture studies (Abramov et al. 2004). Such activated astrocytes are also responsible for the functional failure of neuronal-glial-vascular units. However, the mechanistic details of this astrocyte-mediated failure are still ambiguous, and *Drosophila* models might be helpful in solving the in-depth working of glia in Alzheimer's disease. In addition to the above, the activated microglial cells are also found in association with the amyloid plaques (McGeer and Mcgeer 1995) and cause the inflammatory responses (Heneka and O'Banion 2007). Such inflammatory reactions of microglia and astroglia have been suggested to be intimately associated with the pathogenesis and progression of Alzheimer's disease.

Transgenic expression of human Aβ1-42 fragment (Aβ42) in *Drosophila* results in an age-dependent appearance of several characteristic cellular, molecular, and behavioral phenotypes, typical of the mammalian disease. The phenotypes include Aβ aggregation, neuronal hyperexcitability, mitochondrial dysfunction, impaired locomotion, and reduced life span (Finelli et al. 2004; Crowther et al. 2005; Wentzell and Kretzschmar 2010; Ping et al. 2015). Interestingly, Aβ can also be detected in the glial cells when human Aβ42 is even exclusively expressed in *Drosophila* neurons suggesting that fly glia internalize the secreted Aβ peptides in vivo (Iijima et al. 2008). Another study in the *Drosophila* model of Alzheimer's disease demonstrates *draper* (*Drosophila* ortholog of mammalian MEGF10 and Jedi) as a potential candidate involved in the engulfment of these Aβ peptides by the glial cells. Overexpression of *draper* in glial cells induces protein degradation pathways and engulfing of the misfolded and apoptotic cells, hence providing neuroprotection against Aβ42-induced toxicity (Ray et al. 2017). These findings suggest a significant

involvement of the glial cells in the pathogenesis and progression of Alzheimer's disease.

Frontotemporal Dementia/Non-AD Dementia

Frontotemporal dementia or non-Alzheimer's disease dementia, including several types of sporadic cognitive disruptions such as Pick's disease and frontotemporal lobar degeneration, also display an involvement of activated astroglia and microglia. In several of such diseases, an abundance of tau protein aggregates is observed in the glial cells, which, however, express very little tau protein during normal homeostasis (Komori 1999). The *Drosophila* tauopathy models expressing human tau (h-tau) in the glial cells specifically display an accumulation of hyperphosphorylated tau and formation of tau aggregates in the glial cells. Interestingly, an age-dependent neurodegeneration is also obtained by expressing pathogenic h-tau in glia-specific manner (Colodner and Feany 2010). The severity of dementia positively correlates to the degree of astrocytic degeneration (Kersaitis et al. 2004). Based on above, a larval *Drosophila* model of glial tauopathy was generated which results in larval locomotor deficits, pupal death, and morphological deformities of PNS segmental nerves (Scarpelli et al. 2019). Interestingly, these phenotypes were found to be a consequence of the abnormal accumulation of the presynaptic protein, Bruchpilot (BRP), in the axons (Scarpelli et al. 2019). This model provides insight into the mechanisms leading to the pathogenic effects of glia on tau pathology and glia-neuron interactions in the PNS.

Polyglutamine Diseases

In addition to an imperative involvement of astrocytes in several aggregate-related tauopathies, a similar role has also been suggested for polyglutamine [poly(Q)] diseases (Sofroniew and Vinters 2010; Ben Haim et al. 2015). Some studies suggest that the neuronal damage in Huntington's disease results in astrocyte reactivity, therefore compromising the nourishing and neuroprotective functions of the glial cells (Pekny et al. 2016). Conversely, studies based on rodent models (Shin et al. 2005; Bradford et al. 2009) and human Huntington's disease brain samples (Jansen et al. 2017) suggest that poly(Q)-HTT (huntingtin) may directly affect astrocytes, resulting from their reactivity and contributing to neurodegeneration. In *Drosophila* disease models, it has been demonstrated that glial cells play a non-cell-autonomous role in the pathogenesis of poly(Q) diseases, including Spinocerebellar ataxia-1 (SCA1) and Huntington's disease (Tamura et al. 2009). A similar study in Huntington's disease model when poly(Q)-HTT was selectively expressed in astrocytes shows progressive neurodegeneration and reduction in life span (Bason et al. 2019). It has been observed that DNAJB6 prevents the aggregation of the mutant ATXN3 (SCA3) (Costa and Paulson 2012) and mutant HTT (Hageman et al. 2010; Kakkar et al. 2016); however, whether it works in a cell-autonomous or non-cell-autonomous manner is still enigmatic. However, studies using *Drosophila* model of Huntington's disease show that overexpression of DNAJB6 in astrocytes suppresses the progression of the poly(Q)-HTT in the neurons and extends the life span in a non-cell-autonomous manner (Bason et al. 2019). Similar non-cell-

autonomous effect of the glial DNAJB6 on neurons is also evident in the SCA3 fly model (Bason et al. 2019).

Another study in *Drosophila* demonstrated that downregulation of *egg-derived tyrosine phosphatase* (*edtp*), a homolog of mammalian MTMR14, myotubularin-related protein 14, in glial cells suppresses poly(Q) aggregation and restricts the phenotypic manifestation (Xiao and Qui 2019). Targeted expression of poly (Q) proteins such as HTT and ATXN3 in the glial cells that form the blood-brain barrier and blood-retina barrier of *Drosophila* leads to the impairment of the integrity of these protective barriers suggesting their specific vulnerability to poly(Q)-expanded proteins (Yeh et al. 2018). Hence, studies in the *Drosophila* poly (Q) models indicate toward a major involvement of the glial cells in the pathogenesis of these disorders.

Taken together, the involvement of glial cells in the pathogenesis and progression and/or protection of neurodegenerative disorders is increasingly clear now. Studies performed in the *Drosophila* models of various human neurodegenerative diseases have opened new avenues for research in the field of glia biology and neurodegeneration. Numerous questions which are difficult to address in the mammalian systems can be easily answered by utilization of a biologically simple yet sophisticated *Drosophila* model organism.

7 Concluding Remarks

Several significant novel discoveries during the past decades have established glial cells in the limelight of neuroscience research. Moreover, the role(s) of glial cells have been advanced from sheer supporting cells to key players in the biology of the nervous system. In this chapter, we attempted to summarize the examples from *Drosophila* studies to enlighten that the powerful genetic tools in the fly system can be efficiently applied to investigate the functional diversities of the glial cells, including their potential involvement in neurodegeneration. The notable similarities between *Drosophila* and mammals not only in glial functions but also in molecular pathways designate fly as a prime model organism to unravel the complexities of glial biology. The ever-growing technical promises offered by the *Drosophila* system would not only serve as a discovery tool but also help in establishing the functional significance of different disease-related proteins and their involvement in devastating human neurodegenerative disorders.

Acknowledgment Research programs in the laboratory is supported by grants from the Department of Biotechnology (DBT) and Science and Engineering Research Board (SERB), Government of India, New Delhi, to SS. Pragati, ST, Aqsa, and PA are supported by Lady Tata Memorial Trust, Mumbai, India: UGC-SRF, CSIR-JRF, and DBT-JRF fellowships, respectively. We are grateful to Ms. Nabanita Sarkar for technical support.

References

Abbott NJ (2005) Dynamics of CNS barriers: evolution, differentiation, and modulation. Cell Mol Neurobiol 25:5–23

Abbott NJ, Rönnbäck L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 7:41–53

Abramov AY, Canevari L, Duchen MR (2004) β-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. J Neurosci 24:565–575

Alfonso TB, Jones BW (2002) *gcm2* promotes glial cell differentiation and is required with glial cells missing for macrophage development in *Drosophila*. Dev Biol 248:369–383

Altenhein B (2015) Glial cell progenitors in the *Drosophila* embryo. Glia 63:1291–1302

Altenhein B, Becker A, Busold C, Beckmann B, Hoheisel JD, Technau GM (2006) Expression profiling of glial genes during *Drosophila* embryogenesis. Dev Biol 296:545–560

Altenhein B, Cattenoz PB, Giangrande A (2016) The early life of a fly glial cell. Wiley Interdiscip Rev Dev Biol 5:67–84

Alzheimer A. (1910) Beiträge zur kenntnis der pathologichen Neuroglia und ihrer Beziehungen zu den abbauvorgangen im Nervengewebe. In: Nissl, F., Alzheimer A (Ads.), Histologische und histopathologiche Arbeiten Über die Grosshimrinde mit besonderer Berucksichtingung der pathologischen Anatomie der Geisteskrankheiten. Gustav Fisher, Jena, pp. 401-562.

Augustin H, Grosjean Y, Chen K, Sheng Q, Featherstone DE (2007) Nonvesicular release of glutamate by glial xCT transporters suppresses glutamate receptor clustering in vivo. J Neurosci 27:111–123

Auld VJ, Fetter RD, Broadie K, Goodman CS (1995) Gliotactin, a novel transmembrane protein on peripheral glia, is required to form the blood-nerve barrier in *Drosophila*. Cell 81:757–767

Avet-Rochex A, Kaul AK, Gatt AP, McNeill H, Bateman JM (2012) Concerted control of gliogenesis by InR/TOR and FGF signalling in the *Drosophila* post-embryonic brain. Development 139:2763–2772

Awasaki T, Ito K (2004) Engulfing action of glial cells is required for programmed axon pruning during *Drosophila* metamorphosis. Curr Biol 14:668–677

Awasaki T, Lee T (2011) New tools for the analysis of glial cell biology in *Drosophila*. Glia 59: 1377–1386

Awasaki T, Lai SL, Ito K, Lee T (2008) Organization and postembryonic development of glial cells in the adult central brain of *Drosophila*. J Neurosci 28:13742–13753

Awasaki T, Huang Y, O'Connor MB, Lee T (2011) Glia instruct developmental neuronal remodeling through TGF-β signaling. Nat Neurosci 14:821–823

Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, Iwatsubo T (1998) Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 152:879–884

Bailey AP, Koster G, Guillermier C, Hirst EM, MacRae JI, Lechene CP, Postle AD, Gould AP (2015) Antioxidant role for lipid droplets in a stem cell niche of *Drosophila*. Cell 163:340–353

Bainton RJ, Tsai LT, Schwabe T, DeSalvo M, Gaul U, Heberlein U (2005) Moody encodes two GPCRs that regulate cocaine behaviors and bloodbrain barrier permeability in *Drosophila*. Cell 123:145–156

Barres BA (2008) The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60:430–440

Bason M, Meister-Broekema M, Alberts N, Dijkers P, Bergink S, Sibon OCM, Kampinga HH (2019) Astrocytic expression of the chaperone DNAJB6 results in non-cell autonomous protection in Huntington's disease. Neurobiol Dis 124:108–117

Bauke A-C, Sasse S, Matzat T, Klämbt C (2015) A transcriptional network controlling glial development in the *Drosophila* visual system. Development 142:2184–2193

Baumgartner S, Littleton JT, Broadie K, Bhat MA, Harbecke R, Lengyel JA, Chiquet-Ehrismann R, Prokop A, Bellen HJ (1996) A *Drosophila* neurexin is required for septate junction and blood-nerve barrier formation and function. Cell 87:1059–1068

Beckervordersandforth RM, Rickert C, Altenhein B, Technau GM (2008) Subtypes of glial cells in the *Drosophila* embryonic ventral nerve cord as related to lineage and gene expression. Mech Dev 125:542–557

Ben Haim L, Carrillo-De Sauvage MA, Ceyzeriate K, Escartin C (2015) Elusive roles for reactive astrocytes in neurodegenerative diseases. Front Cell Neurosci 9:278

Berger C, Pallavi SK, Prasad M, Shashidhara LS, Technau GM (2005) A critical role for cyclin E in cell fate determination in the central nervous system of *Drosophila melanogaster*. Nat Cell Biol 7:56–62

Bernardoni R, Vivancos V, Giangrande A (1997) glide/gcm is expressed and required in the scavenger cell lineage. Dev Biol 191:118–130

Beyer K, Ariza A (2007) Protein aggregation mechanisms in synucleinopathies: commonalities and differences. J Neuropathol Exp Neurol 66:965–974

Bhat MA, Rios JC, Lu Y, Garcia-Fresco GP, Ching W, St Martin M, Li J, Einheber S, Chesler M, Rosenbluth J, Salzer JL, Bellen HJ (2001) Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. Neuron 30:369–383

Bittern J, Pogodalla N, Ohm H, Brüser L, Kottmeier R, Schirmeier S, Klämbt C (2020) Neuron-glia interaction in the *Drosophila* nervous system. Dev Neurobiol. https://doi.org/10.1002/dneu.22737

Bliederhaeuser C, Grozdanov V, Speidel A, Zondler L, Ruf WP, Bayer H, Kiechle M, Feiler MS, Freischmidt A, Brenner D, Witting A, Hengerer B, Fändrich M, Ludolph AC, Weishaupt JH, Gillardon F, Danzer KM (2016) Age-dependent defects of alpha-synuclein oligomer uptake in microglia and monocytes. Acta Neuropathol 131:379–391

Booth GE, Kinrade EF, Hidalgo A (2000) Glia maintain follower neuron survival during *Drosophila* CNS development. Development 127:237–244

Borycz J, Borycz JA, Loubani M, Meinertzhagen IA (2002) tan and ebony genes regulate a novel pathway for transmitter metabolism at fly photoreceptor terminals. J Neurosci 22:10549–10557

Borycz J, Borycz JA, Edwards TN, Boulianne GL, Meinertzhagen IA (2012) The metabolism of histamine in the *Drosophila* optic lobe involves an ommatidial pathway: beta-alanine recycles through the retina. J Exp Biol 215:1399–1411

Bossing T, Udolph G, Doe CQ, Technau GM (1996) The embryonic central nervous system lineages of *Drosophila melanogaster*. I. Neuroblast lineages derived from the ventral half of the neuroectoderm. Dev Biol 179:41–64

Braak H, Sastre M, Del Tredici K (2007) Development of alpha-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. Acta Neuropathol 114:231–241

Bradford J, Shin J, Roberts M, Wang CE, Li XJ, Li S (2009) Expression of mutant huntingtin in mouse brain astrocytes causes age-dependent neurological symptoms. Proc Natl Acad Sci USA 106:22,480–22,485

Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118:401–415

Brankatschk M, Dunst S, Nemetschke L, Eaton S (2014) Delivery of circulating lipoproteins to specific neurons in the *Drosophila* brain regulates systemic insulin signaling. Elife 3:e02862

Campbell G, Goring H, Lin T, Spana E, Andersson S, Doe CQ, Tomlinson A (1994) RK2, a glial-specific homeodomain protein required for embryonic nerve cord condensation and viability in *Drosophila*. Development 120:2957–2966

Chaturvedi R, Reddig K, Li HS (2014) Long-distance mechanism of neurotransmitter recycling mediated by glial network facilitates visual function in *Drosophila*. Proc Natl Acad Sci USA 111:2812–2817

Chotard C, Salecker I (2007) Glial cell development and function in the *Drosophila* visual system. Neuron Glia Biol 3:17–25

Collet C, Di Rienzo F, El Hoyek N, Guillot A (2013) Autonomic nervous system correlates in movement observation and motor imagery. Front Hum Neurosci 7:415

Colodner KJ, Feany MB (2010) Glial fibrillary tangles and JAK/STAT-mediated glial and neuronal cell death in a *Drosophila* model of glial tauopathy. J Neurosci 30:16,102–16,113

Costa C, Paulson HI (2012) Progress in neurobiology toward understanding Machado-Joseph disease. Prog Neurobiol 97:239–257

Coutinho-Budd JC, Sheehan AE, Freeman MR (2017) The secreted neurotrophin Spätzle 3 promotes glial morphogenesis and supports neuronal survival and function. Genes Dev 31: 2023–2038

Crews ST (2010) Axon-glial interactions at the *Drosophila* CNS midline. Cell Adh Migr 4:67–71

Crews ST (2019) *Drosophila* embryonic CNS development: neurogenesis, gliogenesis, cell fate, and differentiation. Genetics 213:1111–1144

Crotti A, Benner C, Kerman BE, Gosselin D, Lagier-Tourenne C, Zuccato C, Cattaneo E, Gage FH, Cleveland DW, Glass CK (2014) Mutant Huntingtin promotes autonomous microglia activation via myeloid lineage-determining factors. Nat Neurosci 17:513–521

Crowther DC, Kinghorn KJ, Miranda E, Page R, Curry JA, Duthie FA, Gubb DC, Lomas DA (2005) Intraneuronal Abeta, non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease. Neuroscience 132:123–135

Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1–105

Danjo R, Kawasaki F, Ordway RW (2011) A tripartite synapse model in *Drosophila*. PLoS One 6: e17131

Delgado MG, Oliva C, López E, Ibacache A, Galaz A, Delgado R, Barros LF, Sierralta J (2018) Chaski, a novel *Drosophila* lactate/pyruvate transporter required in glia cells for survival under nutritional stress. Sci Rep 8:1186

DeSalvo MK, Mayer N, Mayer F, Bainton RJ (2011) Physiologic and anatomic characterization of the brain surface glia barrier of *Drosophila*. Glia 59:1322–1340

DeSalvo MK, Hindle SJ, Rusan ZM, Orng S, Eddison M, Halliwill K, Bainton RJ (2014) The *Drosophila* surface glia transcriptome: evolutionary conserved blood-brain barrier processes. Front Neurosci 8:346

Doherty J, Logan MA, Taşdemir OE, Freeman MR (2009) Ensheathing glia function as phagocytes in the adult *Drosophila* brain. J Neurosci 29:4768–4781

Doherty J, Sheehan AE, Bradshaw R, Fox AN, Lu TY, Freeman MR (2014) PI3K signaling and Stat92E converge to modulate glial responsiveness to axonal injury. PLoS Biol 12:e1001985

Dumstrei K, Wang F, Hartenstein V (2003) Role of DE-cadherin in neuroblast proliferation, neural morphogenesis, and axon tract formation in *Drosophila* larval brain development. J Neurosci 23:3325–3335

Dutta S, Rieche F, Eckl N, Duch C, Kretzshmar D (2016) Glial expression of Swiss cheese (SWS), the *Drosophila* orthologue of neuropathy target esterase (NTE), is required for neuronal ensheathment and function. Dis Model Mech 9:283–294

Ebens AJ, Garren H, Cheyette BN, Zipursky SL (1993) The *Drosophila* anachronism locus: a glycoprotein secreted by glia inhibits neuroblast proliferation. Cell 74:15–27

Edwards TN, Meinertzhagen IA (2010) The functional organisation of glia in the adult brain of *Drosophila* and other insects. Prog Neurobiol 90:471–497

Edwards JS, Swales LS, Bate M (1993) The differentiation between neuroglia and connective tissue sheath in insect ganglia revisited: the neural lamella and perineurial sheath cells are absent in a mesodermless mutant of *Drosophila*. J Comp Neurol 333:301–308

Eulenberg KG, Schuh R (1997) The tracheae defective gene encodes a bZIP protein that controls tracheal cell movement during *Drosophila* embryogenesis. EMBO J 16:7156–7165

Farca Luna AJ, Perier M, Seugnet L (2017) Amyloid precursor protein in *Drosophila* glia regulates sleep and genes involved in glutamate recycling. J Neurosci 37:4289–4300

Feany MB, Bender WW (2000) A *Drosophila* model of Parkinson's disease. Nature 404:394–398

Featherstone DE (2011) Glial solute carrier transporters in *Drosophila* and mice. Glia 59:1351–1363

Finelli A, Kelkar A, Song HJ, Yang H, Konsolaki M (2004) A model for studying Alzheimer's Abeta42-induced toxicity in *Drosophila melanogaster*. Mol Cell Neurosci 26:365–375

Flici H, Cattenoz PB, Komonyi O, Laneve P, Erkosar B, Karatas OF, Reichert H, Berzsenyi S, Giangrande A (2014) Interlocked loops trigger lineage specification and stable fates in the *Drosophila* nervous system. Nat Commun 5:4484

Floden AM, Combs CK (2011) Microglia demonstrate age-dependent interaction with amyloid-β fibrils. J Alzheimers Dis 25:279–293

Freeman MR (2015) *Drosophila* central nervous system glia. Cold Spring Harb Perspect Biol 7: a020552

Freeman MR, Doe CQ (2001) Asymmetric Prospero localization is required to generate mixed neuronal/glial lineages in the *Drosophila* CNS. Development 128:4103–4112

Freeman MR, Doherty J (2006) Glial cell biology in *Drosophila* and vertebrates. Trends Neurosci 29:82–90

Freeman MR, Delrow J, Kim J, Johnson E, Doe CQ (2003) Unwrapping glial biology: Gcm target genes regulating glial development, diversification, and function. Neuron 38:567–580

Fuentes-Medel Y, Logan MA, Ashley J, Ataman B, Budnik V, Freeman MR (2009) Glia and muscle sculpt neuromuscular arbors by engulfing destabilized synaptic boutons and shed presynaptic debris. PLoS Biol 7:e1000184

Fuentes-Medel Y, Ashley J, Barria R, Maloney R, Freeman M, Budnik V (2012) Integration of a retrograde signal during synapse formation by glia-secreted TGF-β ligand. Curr Biol 22:1831–1838

Goodman CS, Doe CQ (1993) Embryonic development of the Drosophila central nervous system. In: Bate M, Martines-Arias A (eds) The development of Drosophila melanogaster. Cold Spring Harbor Press, New York, pp 1131–1206

Granderath S, Bunse I, Klämbt C (2000) gcm and pointed synergistically control glial transcription of the *Drosophila* gene loco. Mech Dev 91:197–208

Gratz SJ, Rubinstein CD, Harrison MM, Wildonger J, O'Connor-Giles KM (2015) CRISPR-Cas9 genome editing in *Drosophila*. Curr Protoc Mol Biol 111:31.2.1–31.2.20

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave KA (1998) Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science 280:1610–1613

Hageman J, Rujano MA, van Waarde MA, Kakkar V, Dirks RP, Govorukhina N, Oosterveld-Hut HM, Lubsen NH, Kampinga HH (2010) A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. Mol Cell 37:355–369

Hakim Y, Yaniv SP, Schuldiner O (2014) Astrocytes play a key role in *Drosophil*a mushroom body axon pruning. PLoS One 9:e86178

Harilal S, Jose J, Parambi DGT, Kumar R, Unnikrishnan MK, Uddin MS, Mathew GE, Pratap R, Marathakam A, Mathew B (2020) Revisiting the Blood-brain barrier: a hard nut to crack in the transportation of drug molecules. Brain Res Bull 160:121–140

Hartenstein V (2011) Morphological diversity and development of glia in *Drosophila*. Glia 59: 1237–1252

Hayashi S, Ito K, Sado Y, Taniguchi M, Akimoto A, Takeuchi H, Aigaki T, Matsuzaki F, Nakagoshi H, Tanimura T, Ueda R, Uemura T, Yoshihara M, Goto S (2002) GETDB, a data base compiling expression patterns and molecular locations of a collection of Gal4 enhancer traps. Genesis 34:58–61

Heneka MT, O'Banion MK (2007) Inflammatory processes in Alzheimer's disease. J Neuroimmunol 184:69–91

Heneka MT, Rodriguez JJ, Verkhratsky A (2010) Neuroglia in neurodegeneration. Brain Res Rev 63:189–211

Herculano-Houzel S (2014) The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. Glia 62:1377–1391

von Hilchen CM, Beckervordersandforth RM, Rickert C, Technau GM, Altenhein B (2008) Identity, origin, and migration of peripheral glial cells in the *Drosophila* embryo. Mech Dev 125:337–352

von Hilchen CM, Bustos AE, Giangrande A, Technau GM, Altenhein B (2013) Predetermined embryonic glial cells form the distinct glial sheaths of the Drosophila peripheral nervous system. Development 140:3657–3668

Hindle SJ, Bainton RJ (2014) Barrier mechanisms in the *Drosophila* blood-brain barrier. Front Neurosci 8:414

Hirth F (2010) *Drosophila melanogaster* in the study of human neurodegeneration. CNS Neurol Disord Drug Targets 9:504–523

Hosoya T, Takizawa K, Nitta K, Hotta Y (1995) glial cells missing: a binary switch between neuronal and glial determination in *Drosophila*. Cell 82:1025–1036

Hotta Y, Benzer S (1972) Mapping of behaviour in *Drosophila* mosaics. Nature 240:527–535

Hunter AC, Petley-Ragan LM, Das M, Auld VJ (2020) Basigin associates with integrin in order to regulate perineurial glia and *Drosophila* nervous system morphology. J Neurosci 40:3360–3373

Iijima K, Chiang HC, Hearn SA, Hakker I, Gatt A, Shenton C, Granger L, Leung A, Iijima-Ando K, Zhong Y (2008) Aβ 42 mutants with different aggregation profiles induce distinct pathologies in *Drosophila*. PLoS One 3:e1703

Ismail JN, Badini S, Frey F, Abou-Kheir W, Shirinian M (2019) *Drosophila* tet is expressed in midline glia and is required for proper axonal development. Front Cell Neurosci 13:252

Ito K, Urban J, Technau GM (1995) Distribution, classification, and development of *Drosophila* glial cells in the late embryonic and early larval ventral nerve cord. Roux Arch Dev Biol 204: 284–307

Jacobs JR (2000) The midline glia of *Drosophila*: a molecular genetic model for the developmental functions of glia. Prog Neurobiol 62:475–508

Jansen AH, van Hal M, Op den Kelder IC, Meier RT, de Ruiter AA, Schut MH, Smith DL, Grit C, Brouwer N, Kamphuis W, Boddeke HW, den Dunnen WF, van Roon WM, Bates GP, Hol EM, Reits EA (2017) Frequency of nuclear mutant huntingtin inclusion formation in neurons and glia is cell-type-specific. Glia 65:50–61

Jäkel S, Dimou L (2017) Glial cells and their function in the adult brain: a journey through the history of their ablation. Front Cell Neurosci 11:24

Jenett A, Rubin GM, Ngo TT, Shepherd D, Murphy C, Dionne H, Pfeiffer BD, Cavallaro A, Hall D, Jeter J, Iyer N, Fetter D, Hausenfluck JH, Peng H, Trautman ET, Svirskas RR, Myers EW, Iwinski ZR, Aso Y, DePasquale GM, Enos A, Hulamm P, Lam SC, Li HH, Laverty TR, Long F, Qu L, Murphy SD, Rokicki K, Safford T, Shaw K, Simpson JH, Sowell A, Tae S, Yu Y, Zugates CT (2012) A GAL4-driver line resource for *Drosophila* neurobiology. Cell Rep 2:991–1001

Joers V, Tansey MG, Mulas G, Carta AR (2017) Microglia phenotypes in Parkinson's disease and animal models. Prog Neurobiol 155:57–75

Jones SV, Kounatidis I (2017) Nuclear factor-Kappa B and Alzheimer disease, unifying genetic and environmental risk factors from cell to humans. Front Immunol 8:1805

Jones BW, Fetter RD, Tear G, Goodman CS (1995) glial cells missing: a genetic switch that controls glial versus neuronal fate. Cell 82:1013–1023

Jones BW, Abeysekera M, Galinska J, Jolicoeur EM (2004) Transcriptional control of glial and blood cell development in *Drosophila*: cis-regulatory elements of glial cells missing. Dev Biol 266:374–387

Juang JL, Carlson SD (1994) Analog of vertebrate anionic sites in blood-brain interface of larval *Drosophila*. Cell Tissue Res 277:87–95

Kakkar V, Månsson C, de Mattos EP, Bergink S, van der Zwaag M, van Waarde MAWH, Kloosterhuis NJ, Melki R, van Cruchten RTP, Al-Karadaghi S, Arosio P, Dobson CM, Knowles TPJ, Bates GP, van Deursen JM, Linse S, van de Sluis B, Emanuelsson C, Kampinga HH (2016) The S/T-Rich motif in the DNAJB6 chaperone delays polyglutamine aggregation and onset of disease in a mouse model. Mol Cell 62:272–283

Kanai MI, Kim MJ, Akiyama T, Takemura M, Wharton K, O'Connor MB, Nakato H (2018) Regulation of neuroblast proliferation by surface glia in the *Drosophila* larval brain. Sci Rep 8: 3730

Kazama H, Yaksi E, Wilson RI (2011) Cell death triggers olfactory circuit plasticity via glial signaling in *Drosophila*. J Neurosci 31:7619–7630

Kerr KS, Fuentes-Medel Y, Brewer C, Barria R, Ashley J, Abruzzi KC, Sheehan A, Tasdemir-Yilmaz OE, Freeman MR, Budnik V (2014) Glial wingless/Wnt regulates glutamate receptor clustering and synaptic physiology at the *Drosophila* neuromuscular junction. J Neurosci 34: 2910–2920

Kersaitis C, Halliday GM, Krill JJ (2004) Regional and cellular pathology in frontotemporal dementia: relationship to stage of disease in cases with and without pick bodies. Acta Neurpathol 108:515–523

Kim S, Cho S, Kim KY, Shin KY, Kim HS, Park CH, Chang KA, Lee SH, Cho D, Suh YH (2009) α-Synuclein induces migration of BV-2 microglial cells by up-regulation of CD44 and MT1-MMP 4. J Neurochem 109:1483–1496

Kim SN, Jeibmann A, Halama K, Witte HT, Wälte M, Matzat T, Schillers H, Faber C, Senner V, Paulus W, Klämbt C (2014) ECM stiffness regulates glial migration in *Drosophila* and mammalian glioma models. Development 141:3233–3242

Klaes A, Menne T, Stollewerk A, Scholz H, Klämbt C (1994) The Ets transcription factors encoded by the *Drosophila* gene pointed direct glial cell differentiation in the embryonic CNS. Cell 78: 149–160

Klämbt C, Jacobs JR, Goodman CS (1991) The midline of the *Drosophila* central nervous system: a model for the genetic analysis of cell fate, cell migration, and growth cone guidance. Cell 64: 801–815

Komori T (1999) Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. Brain Pathol 9:663–679

Kremer MC, Jung C, Batelli S, Rubin GM, Gaul U (2017) The glia of the adult *Drosophila* nervous system. Glia 65:606–638

Kretzschmar D, Pflugfelder GO (2002) Glia in development, function, and neurodegeneration of the adult insect brain. Brain Res Bull 57:121–131

Kretzschmar D, Hasan G, Sharma G, Heisenberg M, Benzer S (1997) The Swiss cheese mutant causes glial hyperwraping and brain degeneration in *Drosophila*. J Neurosci 17:7425–7432

Lai SL, Lee T (2006) Genetic mosaic with dual binary transcriptional systems in *Drosophila*. Nat Neurosci 9:703–709

Lebestky T, Chang T, Hartenstein V, Banerjee U (2000) Specification of *Drosophila* hematopoietic lineage by conserved transcription factors. Science 288:146–149

Lee BP, Jones BW (2005) Transcriptional regulation of the *Drosophila* glial gene repo. Mech Dev 122:849–862

Lee T, Luo L (1999) Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22:451–461

Leiserson WM, Harkins EW, Keshishian H (2000) Fray, a *Drosophila* serine/threonine kinase homologous to mammalian PASK, is required for axonal ensheathment. Neuron 28:793–806

Lessing D, Bonini NM (2009) Maintaining the brain: insight into human neurodegeneration from *Drosophila melanogaster* mutants. Nat Rev Genet 10:359–370

Li H-H, Kroll JR, Lennox SM, Ogundeyi O, Jeter J, Depasquale G, Truman JW (2014) A GAL4 driver resource for developmental and behavioral studies on the larval CNS of *Drosophila*. Cell Reports 8:897–908

Limmer S, Weiler A, Volkenhoff A, Babatz F, Klämbt C (2014) The *Drosophila* blood-brain barrier: development and function of a glial endothelium. Front Neurosci 8:365

Lopategui Cabezas I, Herrera Batista A, Pentón Rol G (2014) The role of glial cells in Alzheimer disease: potential therapeutic implications. Neurologia 29:305–309

Losada-Perez M (2018) Glia: from 'just glue' to essential players in complex nervous systems: a comparative view from flies to mammals. J Neurogenet 32:78–91

Lu B, Vogel H (2009) *Drosophila* models of neurodegenerative diseases. Annu Rev Pathol 4:315–342

Lull M, Block ML (2010) Microglial activation and chronic neurodegeneration. Neurotherapeutics 7:354–365

MacNamee SE, Liu KE, Gerhard S, Tran CT, Fetter RD, Cardona A, Tolbert LP, Oland LA (2016) Astrocytic glutamate transport regulates a *Drosophila* CNS synapse that lacks astrocyte ensheathment. J Comp Neurol 524:1979–1998

Matzat T, Sieglitz F, Kottmeier R, Babatz F, Engelen D, Klämbt C (2015) Axonal wrapping in the *Drosophila* PNS is controlled by glia-derived neuregulin homolog Vein. Development 142: 1336–1345

Mayer F, Mayer N, Chinn L, Pinsonneault RL, Kroetz D, Bainton RJ (2009) Evolutionary conservation of vertebrate blood-brain barrier chemoprotective mechanisms in *Drosophila*. J Neurosci 29:3538–3550

McGeer EG, Mcgeer PL (1995) Brain inflammation response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Brain Res Rev 21:195–218

McGeer PL, McGeer EG (2002) Inflammatory processes in amyotrophic lateral sclerosis. Muscle Nerve 26:459–470

McGeer PL, McGeer EG (2008) Glial reactions in Parkinson's disease. Mov Disord 23:474–483

Mederos S, González-Arias C, Perea G (2018) Astrocyte-neuron networks: a multilane highway of signaling for homeostatic brain function. Front Synaptic Neurosci 10:45

Meinertzhagen IA, Hanson TE (1993) The development of the optic lobe. In: BaA M-AM (ed) The development of *Drosophila melanogaster*. Cold Spring Harbor, Cold Spring Harbor Press, pp 1363–1491

Melom JE, Littleton JT (2013) Mutation of a NCKX eliminates glial microdomain calcium oscillations and enhances seizure susceptibility. J Neurosci 33:1169–1178

Mena MA, Garcia de Yebenes J (2008) Glial cells as players in Parkinsonism: the "good", the "bad", and the "mysterious" glia. Neuroscientist 14:544–560

Meyer S, Schmidt I, Klämbt C (2014) Glia ECM interactions are required to shape the *Drosophila* nervous system. Mech Dev 133:105–116

Mohylyak II, Chernyk YI (2017) Functioning of glia and neurodegeneration in *Drosophila melanogaster*. Cytol Genet 51:202–213

Monedero Cobeta I, Salmani BY, Thor S (2017) Anterior-posterior gradient in neural stem and daughter cell proliferation governed by spatial and temporal Hox control. Curr Biol 27:1161–1172

Muthukumar AK, Stork T, Freeman MR (2014) Activity-dependent regulation of astrocyte GAT levels during synaptogenesis. Nat Neurosci 17:1340–1350

Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S (2007) Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat Neurosci 10:615–622

Nagele RG, Wegiel J, Venkataraman V, Imaki H, Wang KC (2004) Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. Neurobiol Aging 25:663–674

Nave KA, Trapp BD (2008) Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci 31:535–561

Nayak D, Roth TL, McGavern DB (2014) Microglia development and function. Annu Rev Immunol 32:367–402

Nolan YM, Sullivan AM, Toulouse A (2013) Parkinson's disease in the nuclear age of neuroinflammation. Trends Mol Med 19:187–196

Novellino F, Saccà V, Donato A, Zaffino P, Spadea MF, Vismara M, Arcidiacono B, Malara N, Presta I, Donato G (2020) Innate immunity: a common denominator between neurodegenerative and neuropsychiatric diseases. Int J Mol Sci 21:1115

Oberheim NA, Wang X, Goldman S, Nedergaard M (2006) Astrocytic complexity distinguishes the human brain. Trends Neurosci 29:547–553

Olsen AL, Feany MB (2019) Glial α-synuclein promotes neurodegeneration characterized by a distinct transcriptional program in vivo. Glia 67:1933–1957

Omoto JJ, Yogi P, Hartenstein V (2015) Origin and development of neuropil glia of the *Drosophila* larval and adult brain: Two distinct glial populations derived from separate progenitors. Dev Biol 404:2–20

Ordonez DG, Lee MK, Feany MB (2018) α-Synuclein induces mitochondrial dysfunction through Spectrin and the actin cytoskeleton. Neuron 97:108–124.e6

Otto N, Marelja Z, Schoofs A, Kranenburg H, Bittern J, Yildirim K, Berh D, Bethke M, Thomas S, Rode S, Risse B, Jiang X, Pankratz M, Leimkühler S, Klämbt C (2018) The sulfite oxidase Shopper controls neuronal activity by regulating glutamate homeostasis in *Drosophila* ensheathing glia. Nat Commun 9:3514

Paladi M, Tepass U (2004) Function of Rho GTPases in embryonic blood cell migration in *Drosophila*. J Cell Sci 117:6313–6326

Pantazis A, Segaran A, Liu CH, Nikolaev A, Rister J, Thum AS, Roeder T, Semenov E, Juusola M, Hardie RC (2008) Distinct roles for two histamine receptors (hclA and hclB) at the *Drosophila* photoreceptor synapse. J Neurosci 28:7250–7259

Peco E, Davla S, Camp D, Stacey M, Landgraf M, van Meyel DJ (2016) *Drosophila* astrocytes cover specific territories of the CNS neuropil and are instructed to differentiate by Prospero, a key effector of Notch. Development 143:1170–1181

Pekny M, Pekna M, Messing A, Steinhäuser C, Lee JM, Parpura V, Hol EM, Sofroniew MV, Verkhratsky A (2016) Astrocytes: a central element in neurological diseases. Acta Neuropathol 131:323–345

Pereanu W, Shy D, Hartenstein V (2005) Morphogenesis and proliferation of the larval brain glia in *Drosophila*. Dev Biol 283:191–203

Pereanu W, Spindler S, Cruz L, Hartenstein V (2007) Tracheal development in the *Drosophila* brain is constrained by glial cells. Dev Biol 302:169–180

Pfeiffer BD, Ngo TT, Hibbard KL, Murphy C, Jenett A, Truman JW, Rubin GM (2010) Refinement of tools for targeted gene expression in *Drosophila*. Genetics 186:735–755

Ping Y, Hahm ET, Waro G, Song Q, Vo-Ba DA, Licursi A, Bao H, Ganoe L, Finch K, Tsunoda S (2015) Linking Aβ 42-induced hyperexcitability to neurodegeneration, learning and motor deficits, and a shorter life span in an Alzheimer's model. PLoS Genet 11:e1005025

Rahman M, Ham H, Liu X, Sugiura Y, Orth K, Kramer H (2012) Visual neurotransmission in *Drosophila* requires expression of Fic in glial capitate projections. Nat Neurosci 15:871–875

Ray A, Speese SD, Logan MA (2017) Glial draper rescues Aβ toxicity in a *Drosophila* model of Alzheimer's disease. J Neurosci 37:11881–11893

Rival T, Soustelle L, Strambi C, Besson MT, Iché M, Birman S (2004) Decreasing glutamate buffering capacity triggers oxidative stress and neuropil degeneration in the *Drosophila* brain. Curr Biol 14:599–605

Rival T, Soustelle L, Cattaert D, Strambi C, Iche M, Birman S (2006) Physiological requirement for the glutamate transporter dEAAT1 at the adult *Drosophila* neuromuscular junction. J Neurobiol 66:1061–1074

Rodriguez JJ, Olabarria M, Chvatal A, Verkhratsky A (2009) Astroglia in dementia and Alzheimer's disease. Cell Death Differ 16:378–385

Romero-Calderón R, Uhlenbrock G, Borycz J, Simon AF, Grygoruk A, Yee SK, Shyer A, Ackerson LC, Maidment NT, Meinertzhagen IA, Hovemann BT, Krantz DE (2008) A glial variant of the vesicular monoamine transporter is required to store histamine in the *Drosophila* visual system. PLoS Genet 4:e1000245

Rosen D, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng H-X, Rahmani Z, Krizus A, McKenna-Yasek D, Cayabyab A, Gaston SM, Berger R, Tanzi RE, Halperin JJ, Herzfeldt B, Van den Bergh R, Hung W-Y, Bird T, Deng G, Mulder DW, Smyth C, Laing NG, Soriano E, Pericak-Vance MA, Haines J, Rouleau GA, Gusella JS, Horvitz HR, Brown RH Jr (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62

Rossi D, Volterra A (2009) Astrocytic dysfunction: insights on the role in neurodegeneration. Brain Res Bull 80:224–232

Sarlus M, Heneka MT (2017) Microglia in Alzheimer's disease. J Clin Invest 127:3240–3249

Sasse S, Neuert H, Klämbt C (2015) Differentiation of *Drosophila* glial cells. Wiley Interdiscip Rev Dev Biol 4:623–636

Scarpelli EM, Trinh VY, Tashnim Z, Krans JL, Keller LC, Colodner KJ (2019) Developmental expression of human tau in *Drosophila melanogaster* glial cells induces motor deficits and disrupts maintenance of PNS axonal integrity, without affecting synapse formation. PloS One 14:e0226380

Schmidt H, Rickert C, Bossing T, Vef O, Urban J, Technau GM (1997) The embryonic central nervous system lineages of *Drosophila melanogaster*. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm. Dev Biol 189:186–204

Schwabe T, Bainton RJ, Fetter RD, Heberlein U, Gaul U (2005) GPCR signaling is required for blood-brain barrier formation in *Drosophila*. Cell 123:133–144

Sepp KJ, Auld VJ (1999) Conversion of lacZ enhancer trap lines to GAL4 lines using targeted transposition in *Drosophila melanogaster*. Genetics 151:1093–1101

Sepp KJ, Schulte J, Auld VJ (2001) Peripheral glia direct axon guidance across the CNS/PNS transition zone. Dev Biol 238:47–63

Shen Y, Wang L, Hirose S, Zhou Z, Liu Q (2018) The transcriptional factor Apt regulates neuroblast differentiation through activating CycE expression. Biochem Biophys Res Commun 499:889–894

Shin JY, Fang ZH, Yu ZX, Wang CE, Li XJ (2005) Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. J Cell Biol 171:1001–1012

Shishido E, Ono N, Kojima T, Saigo K (1997) Requirements of DFR1/Heartless, a mesoderm-specific *Drosophila* FGF-receptor, for the formation of heart, visceral and somatic muscles, and ensheathing of longitudinal axon tracts in CNS. Development 124:2119–2128

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7–35

Song YJC, Halliday GM, Holton JL, Lashley T, O'Sullivan SS, McCann H, Lees AJ, Ozawa T, Williams DR, Lockhart PJ, Revesz TR (2009) Degeneration in different Parkinsonian syndromes relates to astrocyte type and astrocyte protein expression. J Neuropathol Exp Neurol 68:1073–1083

Sonnenfeld MJ, Jacobs JR (1995) Macrophages and glia participate in the removal of apoptotic neurons from the *Drosophila* embryonic nervous system. J Comp Neurol 359:644–652

Soustelle L, Giangrande A (2007) Glial differentiation and the Gcm pathway. Neuron Glia Biol 3: 5–16

Spéder P, Brand AH (2014) Gap junction proteins in the blood-brain barrier control nutrient-dependent reactivation of *Drosophila* neural stem cells. Dev Cell 30:309–321

Spéder P, Brand AH (2018) Systemic and local cues drive neural stem cell niche remodeling during neurogenesis in *Drosophila*. eLife 7:e30413

Spindler SR, Ortiz I, Fung S, Takashima S, Hartenstein V (2009) *Drosophila* cortex and neuropile glia influence secondary axon tract growth, pathfinding, and fasciculation in the developing larval brain. Dev Biol 334:355–368

Spittau B (2017) Aging microglia-phenotypes, functions and implications for age-related neurodegenerative diseases. Front Aging Neurosci 9:194

Stacey SM, Muraro NI, Peco E, Labbé A, Thomas GB, Baines RA, van Meyel DJ (2010) *Drosophila* glial glutamate transporter Eaat1 is regulated by fringe-mediated notch signaling and is essential for larval locomotion. J Neurosci 30:14,446–14,457

Stork T, Engelen D, Krudewig A, Silies M, Bainton RJ, Klämbt C (2008) Organization and function of the blood-brain barrier in *Drosophila*. J Neurosci 28:587–597

Stork T, Bernardos R, Freeman MR (2012) Analysis of glial cell development and function in *Drosophila*. Cold Spring Harb Protoc 1:1–17

Stork T, Sheehan A, Tasdemir-Yilmaz OE, Freeman MR (2014) Neuron-glia interactions through the Heartless FGF receptor signaling pathway mediate morphogenesis of *Drosophila* astrocytes. Neuron 83:388–403

Subramanian A, Siefert M, Banerjee S, Vishal K, Bergmann KA, Curts CCM, Dorr M, Molina C, Fernandes J (2017) Remodeling of peripheral nerve ensheathment during the larval-to-adult transition in *Drosophila*. Dev Neurobiol 77:1144–1160

Suh J, Jackson FR (2007) *Drosophila* ebony activity is required in glia for the circadian regulation of locomotor activity. Neuron 55:435–447

Sun B, Xu P, Salvaterra PM (1999) Dynamic visualization of nervous system in live *Drosophila*. Proc Natl Acad Sci USA 96:10,438–10,443

Tamura T, Sone M, Yamashita M, Wanker EE, Okazawa H (2009) Glial cell lineage expression of mutant ataxin-1 and huntingtin induces developmental and late-onset neuronal pathologies in *Drosophila* models. PloS One 4:e4262

Tasdemir-Yilmaz OE, Freeman MR (2014) Astrocytes engage unique molecular programs to engulf pruned neuronal debris from distinct subsets of neurons. Genes Dev 28:20–33

Taylor JM, Main BS, Crack PJ (2013) Neuroinflammation and oxidative stress: co-conspirators in the pathology of Parkinson's disease. Neurochem Int 62:803–819

Technau GM, Berger C, Urbach R (2006) Generation of cell diversity and segmental pattern in the embryonic central nervous system of *Drosophila*. Dev Dyn 235:861–869

Theodore S, Cao S, McLean PJ, Standaert DG (2008) Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. J Neuropathol Exp Neurol 67:1149–1158

Theodosiou NA, Xu T (1998) Use of FLP/FRT system to study *Drosophila* development. Methods 14:355–365

Thomas GB, van Meyel DJ (2007) The glycosyltransferase Fringe promotes Delta-Notch signaling between neurons and glia, and is required for subtype-specific glial gene expression. Development 134:591–600

Tietz S, Engelhardt B (2015) Brain barriers: crosstalk between complex tight junctions and adherens junctions. J Cell Biol 209:493–506

Udolph G, Prokop A, Bossing T, Technau GM (1993) A common precursor for glia and neurons in the embryonic CNS of *Drosophila* gives rise to segment-specific lineage variants. Development 118:765–775

Unhavaithaya Y, Orr-Weaver TL (2012) Polyploidization of glia in neural development links tissue growth to blood-brain barrier integrity. Genes Dev 26:31–36

Vincent S, Vonesch JL, Giangrande A (1996) Glide directs glial fate commitment and cell fate switch between neurones and glia. Development 122:131–139

Virchow R (1856) Gesammelte E abhandlungen zur Wissenschaftlichen Medicin. Frankfurt a. M, Hamm, p 1024

Virchow R (1858) Cellularpathologie in ihre Begriindung auf Physirr logische und Pathologische Gewebelehre. A. Hirschwald, Berlin, p 440

Volkenhoff A, Weiler A, Letzel M, Stehling M, Klämbt C, Schirmeier S (2015) Glial glycolysis is essential for neuronal survival in *Drosophila*. Cell Metab 22:437–447

Wakabayashi K, Hayashi S, Yoshimoto M, Kudo H, Takahashi H (2000) NACP/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. Acta Neuropathol 99:14–20

Watts RJ, Schuldiner O, Perrino J, Larsen C, Luo L (2004) Glia engulf degenerating axons during developmental axon pruning. Curr Biol 4:678–684

Wentzell J, Kretzschmar D (2010) Alzheimer's disease and tauopathy studies in flies and worms. Neurobiol Dis 40:21–28

Wong AM, Wang JW, Axel R (2002) Spatial representation of the glomerular map in the *Drosophila* protocerebrum. Cell 109:229–241

Xiao C, Qui S (2019) Downregulation of EDTP in glial cells suppresses polyglutamine protein aggregates and extends lifespan in *Drosophila melanogaster*. Neurosci Lett 694:168–175

Xiong WC, Montell C (1995) Defective glia induce neuronal apoptosis in the repo visual system of *Drosophila*. Neuron 14:581–590

Yeh P-A, Liu Y-H, Chu W-C, Liu J-Y, Sun YH (2018) Glial expression of disease-associated polyglutamine proteins impairs the blood-brain barrier in *Drosophila*. Hum Mol Genet 27:2546–2562

Yildirim K, Petri J, Kottmeier R, Klämbt C (2019) *Drosophila* glia: few cell types and many conserved functions. Glia 67:5–26

Zuchero JB, Barres BA (2015) Glia in mammalian development and disease. Development 142:3805–3809

In Vitro Models of Astrocytes: An Overview

Pallavi Pant, Guneet Kaur, and Pankaj Seth

Abstract

Astrocytes are the most abundant cell type in the brain. In recent years, astrocytes have been demonstrated to be important for the physiological functions of the brain and implicated in several neurological disorders. Hence it was necessary to develop appropriate model systems to study the role of astrocytes in the physiological and pathological functions of the brain. Several useful cell culture models have been established to study the cellular and molecular mechanism of the functioning of a healthy and diseased brain. In this chapter, the authors have compiled some of the applicable models of primary cultures of human astrocytes used to study astrocyte-neuron interplay and alterations in the blood-brain barrier as well as the effect of viral proteins in the functions and properties of human astrocytes.

Keywords

Neuroglia cross talk · BMVECs · Blood-brain barrier · TEER · In vitro models

1 Introduction

Astrocytes are star-shaped glial cells that are involved in maintaining the homeostasis of the central nervous system (CNS). Astrocytes participate in almost all the functions of the CNS starting from the development and its role in providing structural and functional support for neurons and for maintaining the physical barrier

P. Pant · G. Kaur · P. Seth (✉)
Cellular and Molecular Neuroscience Division, National Brain Research Centre (NBRC), Gurgaon, Haryana, India
e-mail: pseth.nbrc@gov.in

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8_27

between the blood and brain interface with the support of endothelial cells. Astrocytes also actively engage in neuropathological conditions. A prominent feature exhibited by astrocytes is its competence to alter its morphological features into a reactive state, when presented with homeostatic imbalance. This feature of astrocyte allows the visualization of their normal as well as reactive state providing an advantage in in vitro models. The realization that astrocytes subsist essential role in physiological functions of the CNS motivated neuroscientists to isolate and culture these cells and develop competent in vitro models for its study.

To understand astrocyte biology and its relevance in the CNS, it is critical to design an experimental model that represents the pertinent responses of astrocytes towards physiological as well as pathological conditions. The indispensable aim for an in vitro model is to reduce experimental variables in order to facilitate isolation of cell-specific responses upon experimental treatment. Although in vitro model system can never completely substitute for the in vivo conditions, the model must be designed to offer the closest possible representation of the in vivo conditions. Different models with varied species for astrocyte cultures have been studied for half a century ranging from primary to secondary cell lines from rodent as well as human subjects (Arthur et al. 1987; Behzadian et al. 1995; Cantrill et al. 2012; Takeshita et al. 2014). Most of these cell lines are immortalized by mutations in their genome to allow continuous and indefinite propagation of the astrocytes. Although cell lines are preferred due to their high rate of cell division, the genetic changes can be accumulated with every subsequent passage leading to different phenotypic as well as biochemical changes among the same cell lines conferring potentially varying results (Maqsood et al. 2013). This disadvantage is overcome by the use of primary cells that are not immortalized and contain no manufactured genetic perturbations, thereby presenting with an accurate cellular response of the donor species. Another impediment arising due to the cell lines is their immense high proliferation rate which is impractical in the human body, and thus the molecular quantification from these cell cultures is always an exaggeration of the original physiological conditions. The primary human cell culture allows the study of disease pathogenesis with little or no genetic perturbations of the cell types (Brissette et al. 2013; Lutgen et al. 2020). Moreover, primary cell culture models are important to understand the signaling mechanisms and release of various chemokines and cytokines (Baskin et al. 1997; Hu et al. 2000; Sharma et al. 2007). While the imaging technology of magnetic resonance imaging (MRI) and functional MRI (fMRI) is used in living subjects to gather insights into normal and diseased brain functions at a given time point, it becomes difficult to understand the cellular and molecular dynamics while the disease is progressing or develop methods to halt its further progression.

Astrocytes are involved in various metabolic, inflammatory, and infectious diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and Human immunodeficiency virus (HIV) (Mahar Fatima et al. 2017; Garwood et al. 2011; Lutgen et al. 2020; Mena and García De Yébenes 2008; Vila et al. 2001). The primary hindrance encountered by in vitro studies for understanding the pathological implications of species-specific diseases is the choice of model system. For instance,

several human-specific neuroinfections limit the use of animal models to study the neuropathologies associated with it. One such example is human immunodeficiency virus-1 (HIV-1) infection, which presents not only severe long-term neurological deficits but also a differential expression of the consequent symptoms in humans (Kim et al. 2018). The human-specific infection is one of the principal reasons for the long time it took in understanding the disease pathogenesis and for designing therapeutic strategies of HIV. The leading cause emerges from the failure of translation of rodent model studies into successful clinical trials due to major differences observed in primary cultures and their cell lines. Thus, if not all, at least few similar patterns are expected in human cell line and primary cultures and hence, are preferred over animal models. This is practically evident in the pharmaceutical industries where many a time benchwork is not translated into clinical gain. The primary reason for the difficulty is due to pharmacokinetic, pharmacodynamic, and toxicological properties of the candidate drug that are altered as a result of cell type and species-based biotransformation during the preclinical screening stages (Brandon et al. 2003). The purpose of in vitro cultures is to reproduce as many elements of in vivo physiological conditions, protein expression, permeability, and conductance to understand the structural and functional support contributed by astrocytes. Therefore, human primary cultures portray a better representation of the specific response of a particular cell type which is true for neurological diseases as well.

2 Models for Primary Cell Cultures

The astrocytes are either directly isolated from the adult patients' brain or differentiated from the human fetal neural stem cells (hfNSCs) (Fatima et al. 2016; Gradisnik et al. 2020). In adult patients, the astrocytes are usually isolated during surgery, then maintained in culture conditions for 2–3 weeks, and are later characterized by the expression of glial fibrillary acidic protein (GFAP) (Gradisnik et al. 2020). Another protocol, tedious yet most effective, involves isolation of human neural stem cells from the specific regions of the fetal brain followed by their differentiation into various cell types like neurons and astrocytes to study the effect of viral proteins on neurons and astrocytes or on neuron-glia interactions (Fig. 27.1a) and oligodendrocytes (Mahar Fatima et al. 2017; Priyanka et al. 2020). Moreover, the differentiation, maturation, and response of neural stem cell are region specific. Cells isolated from different regions of the brain have differential protein expression as well as metabolic profiles. The viable cells of immortalized cell lines have a constant control profile that will respond identical throughout a single experimental profile. This is not the case in primary cultures where each cell type of specific region has a specific transcriptional profile that responds differently to the same experimental treatment. Therefore, primary cultures provide us a better picture of the specific response of an experiment in the particular cell type precisely in the brain region and not a cumulative general functional response of the cell (Farmer and Murai 2017).

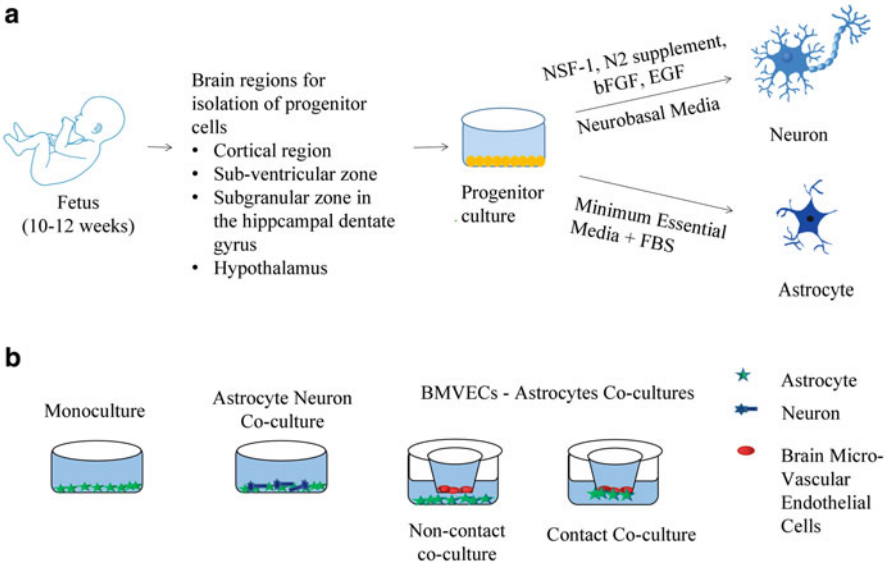

Fig. 27.1 (**a**) Isolation and differentiation of progenitor cells into neurons and astrocytes. Icon credits: https://reactome.org/download-data. (**b**) Different culture methods to study astrocytes

3 Differentiation of Progenitor Cells for In Vitro Cultures

Beside the astrocytic differentiation from hfNSCs isolated from aborted fetus, hfNSCs can be used to understand the effect of various treatments on their differentiation into astrocytes (Fatima et al. 2016). One such model includes the influence of Zika Virus Envelope (E) protein on the differentiation of progenitor cells (Bhagat et al. 2018). The early expression of pro-neuronal genes results in the miRNA regulated NOTCH signaling and immature differentiation of progenitor cells. Also, hfNSCs derived from the telencephalon region have been used to study the components involved in iron homeostasis and its subsequent effect on the iron pool and cell proliferation (Gupta et al. 2019). These models helped to establish the associated phosphorylation and posttranscriptional regulatory role in augmenting the mRNA expression for translation of various genes.

The primary human in vitro models of human neural progenitor cells (hNPCs) help to understand the epigenetic regulation of biological processes. Long noncoding RNAs (lncRNAs) are tissue specific to execute a particular function; thus, a primary culture system provides the specificity required to study their functions (Prajapati et al. 2019). Also, these lncRNAs are developmentally specific and expressed at specific time point in the cell. Thus, autopsy tissues and animal models cannot provide the true understanding of their involvement in transient gene expression and the subsequent functions. The correct correlation of sequencing datasets is validated best in primary cells. An example involving the epigenetic markers of

transcriptional activation (H3K4me3) and inhibition (H3K27me3) had the expected association with divergent lncRNA expression in hNPCs and differentiated cells. Moreover, the pathological effects of infectious diseases like Zika virus are presented as a consequence of microcephaly in the fetus of an infected mother. The mechanism by which this effect is exhibited lacks clarity because the progression of a disease involves crossing placental barrier and blood-brain barrier (BBB) for the virus to reach the fetal brain from infected mother. Due to various such variables, a genetically different cell culture (primary cell line/mouse/secondary cell line) is an added disadvantage that provides a gap. Primary human progenitor cell model eliminates such disadvantages and allows the identification of the effect of Zika virus on the fetal brain as well as its interaction with neural stem cells and how it affects their differentiation into neurons or glial cells. One such study investigated the role of Envelope protein (E-protein) of Zika virus to halt the NSCs in quiescence stage of G0-G1 phase in cell cycle (Bhagat et al. 2018). As a result, apoptosis is induced in differentiating NSCs with reduced migration of cells, perhaps causing microcephaly in fetus. The effect of Zika viral proteins on properties of human astrocytes are being studied at the National Brain Research Centre. This is especially important as astrocytes influence neuronal function.

4 In Vitro Models for Astrocyte Monoculture

Primary human astrocytes are derived from progenitor cells isolated from aborted fetuses. The differentiation of progenitor cells takes approximately 21 days to form mature astrocytes that are usually characterized by the positive expression of GFAP, S100β, and aldehyde dehydrogenase 1 (ALDH1) (Fig. 27.1b) (Fatima et al. 2017; Priyanka et al. 2020). The release of various factors during the differentiation of astrocytes can be investigated through analysis of protein expression in cells and cell-free supernatant. This method helped to identify the production of C-C motif chemokine ligand 2 (CCL2) chemokine at different time points of astrocyte differentiation (Lawrence et al. 2006; Oh et al. 1999). Astrocytes are known for their wide range of involvement in supporting the survival and function of neurons (Pandey and Seth 2019). One model system to identify the numerous properties of astrocytes involves the seeding of the cells on culture plates and incubating the media with the experimental stimulations. The interleukin-1β (IL-1β) cytokine stimulation results in inflammatory response of astrocytes which is effectively decreased through flavonoids by decreasing the release of nitric oxide (NO), reactive oxygen species (ROS), IL-6, IL-8, *Monocyte chemoattractant protein-1* (MCP-1), and regulated upon activation, normal T Cell expressed and presumably secreted (RANTES) (Abdel-Haq et al. 1999; Hu et al. 1995; Sharma et al. 2007). Another association identified by IL-1β stimulation is the glutamate uptake activity (Hu et al. 2000). IL-1β and tumor necrosis factor-α (TNF-α) decrease the glutamate uptake activity of astrocytes, while interferon-γ (IFN-γ) has a counter effect to stimulate its uptake. Such systems can be replicated to understand the inflammatory role of astrocytes in various infectious and inflammatory disorders.

Astrocytes are not only involved in maintaining the homeostasis of the CNS but also play a significant role in disease pathogenesis. It is involved in the progression of AD with a very high amyloidogenic processing of amyloid precursor protein (APP) to promote neurodegeneration in the aging brain (LeBlanc et al. 1997). The human brain cells can be harvested from fetus followed by seeding, maintenance and isolation in culture flasks wherein the microglia are detached through gentle shaking of mixed-glia cultures and astrocytes are purified by vigorous shaking, while the proliferating cells are chemically inhibited. Another in vitro disease model of astrocytes involves infection with HIV to specify the role of astrocytes in its progression (Lutgen et al. 2020; Rojas-Celis et al. 2019). These cells act as cell reservoir that provides latency to the virus and may aid in generating neurological complications and egression of the virus to the peripheral systems. Although the mechanism of infection of astrocytes is still unclear, the HIV infection of normal human astrocytes induces an increased expression of toll-like receptor (TLR) 3, 4, and 5 that further induces the expression and secretion of pro-inflammatory cytokines like TNF-α, IL-6, and IL-8 which causes reduced apolipoprotein E (ApoE) secretions in normal astrocytes. This causes increased reactivation of astrocytes characterized by increased expression of GFAP (Baskin et al. 1997; Serramía et al. 2015). Hence, primary human astrocytes provide critical contribution in determining physiological as well as pathological conditions of the central nervous system.

5 In Vitro Models for Astrocyte-Neuron Interactions

The astrocytes are closely associated with neurons and play an essential role in their physiological functions including neurotransmitter release and uptake, maintenance of pH, and ionic as well as metabolic homeostasis (Pandey and Seth 2019) (Parpura and Verkhratsky 2012). Thus, it is important to study the interaction of astrocytes with the neurons and how the cell types communicate to maintain the homeostasis in the CNS. As astrocytes release various crucial factors, understanding the influence of individual factors requires a robust system. Different in vitro models with more advanced approach have been established to study this relationship. One such model developed by us includes cultures of the astrocytes and neurons separately followed by treatment with viral protein or transfection of astrocytes with gene of interest. Subsequently, at defined time points, conditioned media (containing secretory products from astrocytes in response to the treatment) are collected. This is later added to neuronal cultures to study the effect of viral protein on astrocyte and its consequence on neuronal health. This allows the treatment of neurons with the conditioned media that consist of unknown substances secreted by treated astrocytes in the media that may have the potential to stimulate neurons. Another protocol involves the coculture of astrocytes and neurons to study their interaction. First the neural stem cells are differentiated into neurons and astrocytes separately in the dish/flasks. After the cells have matured, astrocytes are de-adhered from the flask and transferred to the neuronal flask and are allowed to attach to the surface. As

astrocytes always outnumber the neurons, astrocytes are always plated at least double in number to the neurons to keep the model physiologically relevant.

The cross talk between two cell types is a complex interaction that involves release of various soluble factors from one cell type released in extracellular space that acts as a stimulus for another. The degeneration of neurons is the major contributing factor in neuroinflammation and disease pathogenesis. As neuroinflammation is a complex mechanism that tries to maintain homeostasis in the brain, an imbalance toward the pro-inflammatory stimuli causes over-excitation of the glial cells and the subsequent initiation of an inflammation cycle that affects not only the cell itself but the surrounding cells. Neurons are not isolated cell types in the CNS but exist with other cells in its immediacy including astrocytes, oligodendrocytes and microglia. They interact with each other, and the subsequent response can be studied through in vitro cells treated with conditioned media, as in the case of studies on the miRNA and its regulatory signaling pathways. In neuroAIDS, HIV-1 protein, transactivator of transcription (Tat) is detected in the cerebrospinal fluid of infected patients (Mahar Fatima et al. 2017; Priyanka et al. 2020). The media collected from HIV-1 Tat transfected astrocytes when added to neuronal cultures resulted in neuron death through apoptosis (Mahar Fatima et al. 2017). HIV-1 Tat transfected astrocyte conditioned media were used to study the association of astrocytes with the neurons. Using primary cultures of human astrocytes, it was observed that miR-320a is downregulated upon HIV-1 Tat transfection and causes upregulation of its potential target, voltage-dependent anion-selective channel 1 (VDAC1). Upregulation of VDAC1 facilitates the extracellular release of ATP in high concentrations. Thus, astrocytes influence neuronal activity in HIV patients through miR-320a mediated upregulation of VDAC1 to release ATP in the extracellular milieu that causes neurodegeneration.

A study using primary cultures of human astrocytes revealed that neurotoxic role of HIV-1 Tat is rescued by mortalin mediated degradation of HIV-1 Tat through direct binding, that prevented HIV-1 Tat mediated neuronal death (Priyanka et al. 2020). Collectively these studies with human astrocytes helped in delineating mechanisms for neurodegenerative as well as neuroprotective response of HIV-1 Tat transfected astrocytes. Similarly, conditioned media in vitro models aid to examine the effect of various chemical compounds for their therapeutic advancements. Studies conducted using human astrocytes have helped in understanding cellular mechanisms of Alzheimer's disease. The conditioned medium from oligomeric amyloid beta-treated human astrocytes induces cell death in the primary culture of human neurons, which is inhibited by sinomenine incubation (Singh et al. 2020). The sinomenine conferred neuroprotection to hippocampal neurons by inhibiting the production of amyloid beta-induced production of toxic factors by the astrocytes.

Astrocytes have an indispensable influence on the neuronal activity due to its proximity with the neurons and other glial cells. Astrocytes forms a tripartite synapse with pre- and postsynaptic neurons at the synapse and, therefore, influence the interaction between the two neurons (Fig. 27.2). The in vitro models developed to study the tripartite synapse include physiological as well as pathological aided

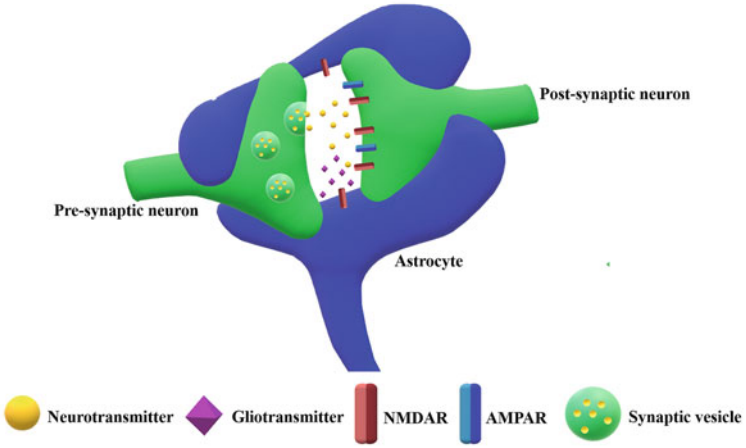

Fig. 27.2 Astrocyte establishes tripartite synapse with pre- and postsynaptic neurons

conditions. During synapse formation, transmission and plasticity is regulated by astrocytes which facilitate supply of enhanced D-serine to N-methyl-D-aspartate (NMDA) receptors (Halassa et al. 2007).

The association of astrocytes with the neurons is also studied in coculture in vitro models of pathological conditions like AIDS and Alzheimer's disease to name a few (Mahar Fatima et al. 2017; Garwood et al. 2011; Lutgen et al. 2020; Mena and García De Yébenes 2008; Vila et al. 2001). The astrocyte-neuron communication is not limited to extracellular matrix mediated stimulation but also physical contact between them. So, in order to remove this barrier, cocultures of astrocyte and neurons are used to maintain a physical contact between the cells. La Crosse virus (LACV) is known to cause neuropathologies including meningitis and encephalitis as a result of inflammation caused after the virus enters through olfactory bulb. As the disease involves lymphocyte infiltration along with inflammation, astrocytes are also observed to be infected. As the pathogenic mechanism is unknown, the use of a coculture model to study the virus is warranted. One model developed involves coculture of astrocytes and neurons differentiated from hNSCs and its subsequent response on viral infection (Dawes et al. 2018). The coculture was then infected with LACV to check the susceptibility of neurons as well as neurons using various biochemical assays like cytotoxicity, apoptosis, and MMP activity assay. The virus-infected coculture not only demonstrates cell-specific vulnerability of the infection but also replicates key aspects of in vivo infection quite accurately.

6 In Vitro Models for Communication of Astrocyte-Brain Microvascular Endothelial Cells

Apart from the close proximity of astrocytes with neurons and other glial cells in the CNS, astrocytes have the first point of contact with vascular endothelial cells of circulatory system as well. The astrocytes along with endothelial cells and pericytes form an interface between the CNS and peripheral circulation to build a neurovascular unit (NVU). This interface, called the blood-brain barrier (BBB), protects the brain from indigenous or exogenous harmful agents. Astrocytes maintain a close association with the systemic circulation for functional partnership. The interaction of astrocytes and brain microvascular endothelial cells (BMVECs) is a two-way communication where astrocytes induce expression of various proteins including tight junction proteins on the BMVECs and vice versa to respond to the constantly changing environment inside as well as outside the CNS (Arthur et al. 1987).

As the replication of in vivo characteristics of the cells into the in vitro systems is defined by the cell-type origins, the primary cells play an essential role in determining the results of the model systems. Primary cells isolated from species other than humans have been used to replicate the in vivo BBB, while primary cocultures of endothelial cells and astrocytes have been used with semipermeable filter to study the morphology, functional markers, electrical resistance, and paracellular transport of molecules (Cantrill et al. 2012; Gaillard et al. 2001; Harris et al. 2010). The cocultures of astrocytes and endothelial cells provide a comparable electrical resistance to those in vivo models of BBB. Although the cells remain in close proximity with each other, a physical contact is not established when primary human cells are cocultured together implying that the interaction probably takes place through secreted factors (Siddharthan et al. 2007). Human fetal astrocytes induce a decreased permeability and increased resistance in the human BMVECs (derived from adults and children). As the astrocytes help to maintain the barrier properties of BMVECs, an in vitro model was established to replicate in vivo conditions of sheer stress by blood flow experienced by BMVECs (Fig. 27.3a). On the apical side of BMVECs in the transwell, the cells were exposed to silicon tube connected to a pump while the basolateral side is exposed to astrocytes. An increased sheer stress causes upregulation of tight junction protein Zonula occludens-1 (ZO-1) which is the reason for decreased permeability and increased resistance induced by astrocytes in BMVECs.

Due to lack of sufficient knowledge of BBB, it is challenging to establish an in vitro model that completely replicates the in vivo system. Yet, in vitro models are designed and validated through various markers for the functional proteins, enzymes, receptors, and transporters (Helms et al. 2016). Apart from the expression of adherens and tight junction proteins, one such validation marker is transendothelial electrical resistance (TEER) that measures the junctional tightness between the endothelial cells by measuring the electrical resistance across the cells. To measure this electrical property of endothelial cells, a transwell set is used in which the endothelial cells are seeded at the luminal (blood) side and resistance is

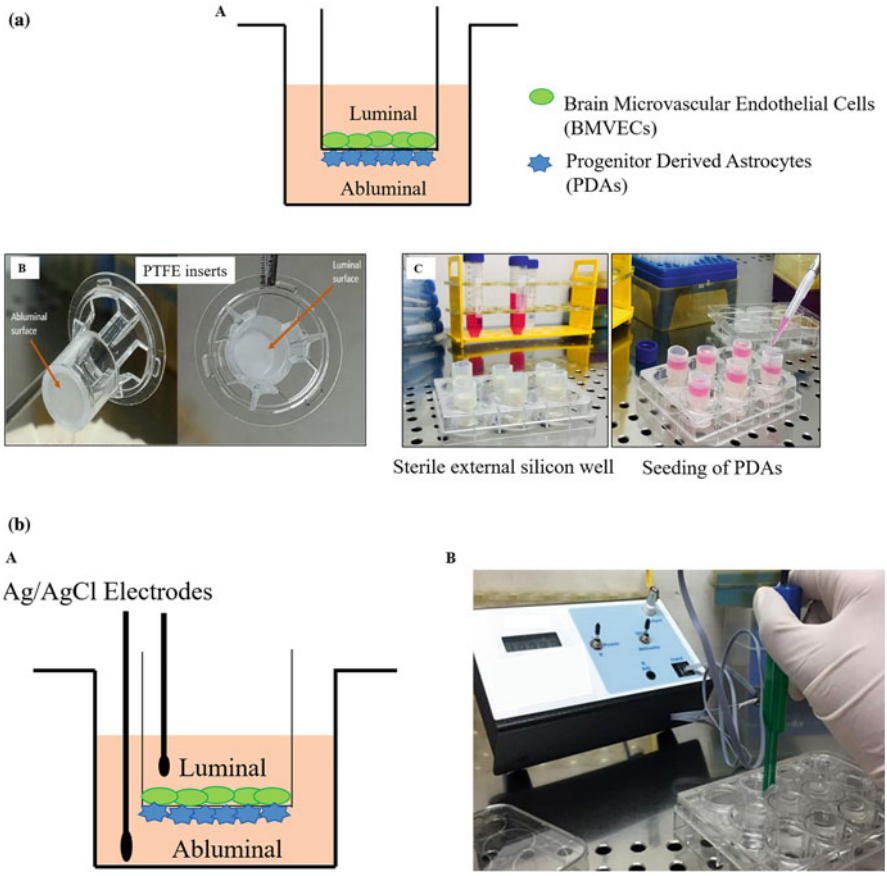

Fig. 27.3 (**a**) **Establishment of contact-based coculture of human blood-brain barrier.** (**A**) shows schematic diagram of contact-based coculture of human blood-brain barrier. (**B**, **C**) depicts the assembly and coculture of BMVECs and PDAs on polytetrafluoroethylene (PTFE) membrane (3 um pore, 12 mm diameter) transwell inserts. (**b**) **Validation of two-dimensional BBB model using TEER.** (**A**) shows schematic diagram of electrodes of the equipment. (**B**) depicts the TEER meter in use

measured through electrodes connected to electrical equipment. The TEER provides the information on paracellular permeability that helps to determine the integrity of BBB (Fig. 27.3b). As the endothelial cells also experience sheer stress due to blood flow, bovine-derived endothelial cells monocultured and cocultured with glial cells show significant differences possibly due to expression of mechano-sensitive channels in astrocytes (Cucullo et al. 2002; Wanner 2012). The cultures attached to an artificial hollow pipe with regulated liquid flow are utilized to measure the difference in the permeability of the endothelial cells. Coculture models exhibit an elevated TEER implying the important role of glial cells in providing resistance,

selective permeability, and thus the integrity of BBB. A similar experimental design is used with human cell lines of endothelial cells and astrocytes that show increased leukocyte migration and functional response of BBB (Takeshita et al. 2014). These experimental models, if implemented with human primary cells, can serve as a competent system that can be extensively employed in pharmacological, morphological, and physiological studies. Another transwell configuration utilizes a gelatin-coated transparent polyester (PET) membrane at the bottom of which cells are seeded to study the electrical resistance provided by BMVECs (Kuo and Lu 2011). The transparent PET membrane is not only biocompatible but also removes the drawback of optical obstruction usually presented with transwell models. This transwell configuration can also be used to study the effects of conditioned media. The model is further improvised by placing the transwell on a plate containing conditioned media, or a different cell type can be seeded on either the abluminal (brain) side of transwell or the culture plate. This is beneficial to study permeability parameters of the BBB and the drug delivery efficiency to the CNS. Further, the porous membrane can also be coated with matrix in order to maintain the cell attachment as well extracellular signaling. One such method includes a Geltrex composition that includes laminin, collagen IV, heparin sulfate proteoglycans, and entactin/nidogen, representing a more in vivo-like extracellular matrix (Lauranzano et al. 2019). This microfluidic membrane model can be used to study not just the electrical resistance or permeability but also ion influx, paracellular interactions, and functional response to inflammatory stimuli. To allow greater extent of advancement, high-quality and mass-production in vitro models of BBB need to be established using primary human endothelial cells and astrocytes for the efficient operation of physiological, pharmacological, and pathophysiological behavior.

7 Induced Pluripotent Cell (iPSC) Models of Astrocyte Cultures

Neural stem cells are categorized by their potential of self-renewability, ability to generate a large number of progeny, and the ability to differentiate into neurons, astrocytes, or oligodendrocytes (Yan et al. 2013; Kuijlaars et al. 2020; Yuan et al. 2013). As isolation and procurement of primary human neural stem cells is difficult and time-consuming, another model system was established that induces the cell fate of human pluripotent cells to neural stem cells or differentiation into CNS cell types. Human induced pluripotent stem cells (iPSCs) derived from patients provide a better picture of neuropathologies. One model to study the interaction of astrocytes and neurons is an in vitro model of human iPSC-derived cortical neurons and human primary astrocytes providing a fully humanized in vitro system (Kuijlaars et al. 2020). The iPSC-derived cell culture models facilitate the growth of therapeutic regenerative medicine for traumatic injury and neurodegeneration (Ottoboni et al. 2017) (Fernandes and Chari 2016). The iPSC-derived hNSCs are characterized by positive expression of Pax6, Sox1, Sox2, and Nestin and negative for Oct4 (Yan et al. 2013). The reprogramming of iPSCs into NSCs is time preserving and

constitutes an efficient method that can be utilized in cell therapy and other regenerative medicine. Peripheral blood mononuclear cells (PBMCs) harvested from human subjects can be used to generate neural stem cells (Channakkar et al. 2020). The PBMCs are first reprogrammed into iPSCs which can further differentiate human induced neural stem cells (hiNSCs) characterized by the positive expression of SOX2 and Nestin. This hiNSC generation is regulated by the ectopic expression of miR-137 which reduces the proliferation rate of hiNSCs. Also, it accelerates neuronal differentiation and migration through a feed-forward self-regulatory loop between miR-132 and OCT4/SOX2 to determine NSC fate. Another method to use induced culture cell-type technology is by inducing the expression of specific transcription factors that reprogram the fate of astrocytes into neurons. The transcription factors activate various cell fate determination pathways including Shh, Wnt, and TGF signaling pathways. One such method includes the induction of primary human astrocytes to form neurons (Rivetti et al. 2017). The model utilized three transcription factors—NEUROD1, ASCL1 and LMX1A, and the microRNA miR-218, collectively designated NeAL218—for the induction of dopaminergic neurons from primary human astrocytes. The induced reprograming of somatic cells from normal donors and patients is comparably easy and posed restricted ethical issues for its formation and hence, is considered an excellent methodology to study disease pathogenesis.

8 3D Models Used for Astrocytes

As the microenvironment of astrocytes includes the cellular system as well as an intricate and complex organ level, it is imperative to establish a model that accounts for the spatial specificity and cell-type networking to study the physiological and pathological response of the astrocytes in the brain. Also, when a toxic response is generated by a cell, its effect is not localized but systemic. An unregulated expression and release of free radicals containing reactive species causes a tissue-wide damage not restricted to specific cell types (Oyefeso et al. 2021). This systemic effect of toxins is mediated via the extracellular matrix (ECM) which plays an essential role in the delivery of stimuli to maintain proper communication between the cells in physiological conditions. So, it is critical to account the importance of ECM for astrocyte response. One such model includes a 3D matrix consisting of collagen I, hyaluronic acid (HA), and Matrigel to study their interaction with the ECM (Placone et al. 2015). The concentration of each component can vary depending upon the experimenter and the environment required to study the astrocyte response, which influences the stiffness and relaxation of gel. Therefore, this allows the varied elastic and sheer modulus of astrocytes. This is established by seeding the astrocytes on the gel followed by 3D microscopy. This provides a good visualization of cell morphology and also the influence of physical factors on the cell morphology. As these gels are compatible with human BMVECs as well, they can be further used to study the physiology of BBB involving astrocytes in addition. As 86 billion neuronal and 85 billion nonneuronal cells exist in the ECM to form a tissue and organ, 3D model

studies have eventually been developed as a savior to understand the astrocytes in the brain as a part of it and not in isolation.

9 Conclusion

Given the importance of astrocytes in the maintenance of brain homeostasis and overall brain functions, detailed studies are required to understand the intricacies of neuron-glia cross talk. As debated in this chapter, animal models and immortalized cell lines often fail to truly represent the normal and diseased brain conditions. Hence, an apt and robust model system is needed. Much of our understanding regarding the glial-neural interplay as well as about the physiological roles of astrocytes comes from experiments done with human astrocytes and cell culture models. Use of primary astrocytes is unarguably a better choice; however, due to technological challenges associated with primary cultures of human cells, neuroscientists often rely on immortalized cell lines. In this chapter, authors list several research findings that helped in deciphering role of astrocytes in neuropathogenesis.

References

Abdel-Haq N, Hao HN, Lyman WD (1999) Cytokine regulation of CD40 expression in fetal human astrocyte cultures. J Neuroimmunol 101(1):7–14. https://doi.org/10.1016/S0165-5728(99)00124-1

Arthur FE, Shivers RR, Bowman PD (1987) Astrocyte-mediated induction of tight junctions in brain capillary endothelium: an efficient in vitro model. Brain Res 36:155–159

Baskin F, Smith GM, Fosmire JA, Rosenberg RN (1997) Altered apolipoprotein E secretion in cytokine treated human astrocyte cultures. J Neurol Sci 148(1):15–18. https://doi.org/10.1016/S0022-510X(96)05335-X

Behzadian MA, Wang X-L, Jiang B, Caldwell RB (1995) Angiostatic role or astrocytes: suppression of vascular endothelial cell growth by TGF-β and other inhibitory factor(s). Glia 15(4):480–490. https://doi.org/10.1002/glia.440150411

Bhagat R, Prajapati B, Narwal S, Agnihotri N, Adlakha YK, Sen J, Mani S, Seth P (2018) Zika virus E protein alters the properties of human fetal neural stem cells by modulating microRNA circuitry. Cell Death Differ 25(10):1837–1854. https://doi.org/10.1038/s41418-018-0163-y

Brandon EFA, Raap CD, Meijerman I, Beijnen JH, Schellens JHM (2003) An update on in vitro test methods in human hepatic drug biotransformation research: pros and cons. Toxicol Appl Pharmacol 189:233–246. https://doi.org/10.1016/S0041-008X(03)00128-5

Brissette CA, Kees ED, Burke MM, Gaultney RA, Floden AM, Watt JA (2013) The multifaceted responses of primary human astrocytes and brain microvascular endothelial cells to the Lyme disease spirochete. Borrelia Burgdorferi 5(3):221–230. https://doi.org/10.1042/AN20130010

Cantrill CA, Skinner RA, Rothwell NJ, Penny JI (2012) An immortalised astrocyte cell line maintains the in vivo phenotype of a primary porcine in vitro blood—brain barrier model. Brain Res 1479:17–30. https://doi.org/10.1016/j.brainres.2012.08.031

Channakkar AS, Singh T, Pattnaik B, Gupta K, Seth P, Adlakha YK (2020) MiRNA-137-mediated modulation of mitochondrial dynamics regulates human neural stem cell fate. Stem Cells 38(5):683–697. https://doi.org/10.1002/stem.3155

Cucullo L, Mcallister MS, Kight K, Krizanac-bengez L, Marroni M, Mayberg MR, Stanness KA, Janigro D (2002) A new dynamic in vitro model for the multidimensional study of astrocyte—endothelial cell interactions at the blood—brain barrier. Brain Res 951:243–254

Dawes BE, Gao J, Atkins C, Nelson JT, Johnson K, Wu P, Freiberg AN (2018) Human neural stem cell-derived neuron/astrocyte co-cultures respond to La Crosse virus infection with proinflammatory cytokines and chemokines. J Neuroinflammation 5:1–15

Farmer WT, Murai K (2017) Resolving astrocyte heterogeneity in the CNS. Front Cell Neurosci 11 (September):1–7. https://doi.org/10.3389/fncel.2017.00300

Fatima M, Kumari R, Schwamborn JC, Mahadevan A, Shankar SK, Raja R, Seth P (2016) Tripartite containing motif 32 modulates proliferation of human neural precursor cells in HIV-1 neurodegeneration. Cell Death Differ 23(5):776–786. https://doi.org/10.1038/cdd.2015.138

Fatima M, Prajapati B, Saleem K, Kumari R, Mohindar Singh Singal C, Seth P (2017) Novel insights into role of miR-320a-VDAC1 axis in astrocyte-mediated neuronal damage in neuroAIDS. Glia 65(2):250–263. https://doi.org/10.1002/glia.23089

Fernandes AR, Chari DM (2016) Part II: functional delivery of a neurotherapeutic gene to neural stem cells using minicircle DNA and nanoparticles: translational advantages for regenerative neurology. J Control Release 238:300–310. https://doi.org/10.1016/j.jconrel.2016.06.039

Gaillard PJ, Voorwinden LH, Nielsen JL, Ivanov A, Atsumi R, Engman H, Ringbom C, De Boer AG, Breimer DD (2001) Establishment and functional characterization of an in vitro model of the blood–brain barrier, comprising a co-culture of brain capillary endothelial cells and astrocytes. Eur J Pharm Sci 12:215–222

Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W (2011) Astrocytes are important mediators of Aβ-induced neurotoxicity and tau phosphorylation in primary culture. Cell Death Disease 2(6):1–9. https://doi.org/10.1038/cddis.2011.50

Gradisnik L, Maver U, Bosnjak R, Velnar T (2020) Optimised isolation and characterisation of adult human astrocytes from neurotrauma patients. J Neurosci Methods 341(March):108796. https://doi.org/10.1016/j.jneumeth.2020.108796

Gupta P, Singh P, Pandey HS, Seth P, Mukhopadhyay CK (2019) Phosphoinositide-3-kinase inhibition elevates ferritin level resulting depletion of labile iron pool and blocking of glioma cell proliferation. Biochim Biophys Acta Gen Subj 1863(3):547–564. https://doi.org/10.1016/j.bbagen.2018.12.013

Halassa MM, Fellin T, Haydon PG (2007) The tripartite synapse: roles for gliotransmission in health and disease. Trends Mol Med 13(2):54–63. https://doi.org/10.1016/j.molmed.2006.12.005

Harris CA, Resau JH, Hudson EA, West RA, Moon C, Mcallister JP (2010) Mechanical contributions to astrocyte adhesion using a novel in vitro model of catheter obstruction. Exp Neurol 222(2):204–210. https://doi.org/10.1016/j.expneurol.2009.12.027

Helms HC, Abbott NJ, Burek M, Cecchelli R, Couraud P, Deli MA, Galla HJ, Romero IA, Shusta EV, Stebbins MJ, Vandenhaute E, Weksler B, Brodin B (2016) In vitro models of the blood–brain barrier: an overview of commonly used brain endothelial cell culture models and guidelines for their use. J Cereb Blood Flow Metab 36:862–890. https://doi.org/10.1177/0271678X16630991

Hu S, Sheng WS, Peterson PK, Chao CC (1995) Differential regulation by cytokines of human astrocyte nitric oxide production. Glia 15(4):491–494. https://doi.org/10.1002/glia.440150412

Hu S, Sheng WS, Ehrlich LC, Peterson PK, Chao CC (2000) Cytokine effects on glutamate uptake by human astrocytes. Neuroimmunomodulation 7(3):153–159. https://doi.org/10.1159/000026433

Kim S, Hahn YK, Podhaizer EM, McLane VD, Zou S, Hauser KF, Knapp PE (2018) A central role for glial CCR5 in directing the neuropathological interactions of HIV-1 tat and opiates. J Neuroinflammation 15(1):1–15. https://doi.org/10.1186/s12974-018-1320-4

Kuijlaars J, Oyelami T, Diels A, Rohrbacher J, Versweyveld S, Meneghello G, Tueffer M, Verstraelen P, Detrez JR, Verschuuren M, De Vos WH, Meert T, Peeters PJ (2020) Sustained

synchronized neuronal network activity in a human astrocyte co-culture system. Sci Rep 6:1–14. https://doi.org/10.1038/srep36529

Kuo Y, Lu C (2011) Colloids and surfaces B: biointerfaces effect of human astrocytes on the characteristics of human brain-microvascular endothelial cells in the blood–brain barrier. Colloids Surf B: Biointerfaces 86(1):225–231. https://doi.org/10.1016/j.colsurfb.2011.04.005

Lauranzano E, Campo E, Rasile M, Molteni R, Pizzocri M, Passoni L, Bello L, Pozzi D, Pardi R, Matteoli M, Ruiz-moreno A (2019) A microfluidic human model of blood–brain barrier employing primary human astrocytes. Adv Biosyst 3:e1800335. https://doi.org/10.1002/adbi.201800335

Lawrence DMP, Seth P, Durham L, Diaz F, Boursiquot R, Ransohoff RM, Major EO (2006) Astrocyte differentiation selectively upregulates CCL2/monocyte chemoattractant protein-1 in cultured human brain-derived progenitor cells. Glia 53(1):81–91. https://doi.org/10.1002/glia.20261

LeBlanc AC, Papadopoulos M, Bélair C, Chu W, Crosato M, Powell J, Goodyer CG (1997) Processing of amyloid precursor protein in human primary neuron and astrocyte cultures. J Neurochem 68(3):1183–1190. https://doi.org/10.1046/j.1471-4159.1997.68031183.x

Lutgen V, Narasipura SD, Barbian HJ, Richards M, Wallace J, Razmpour R, Buzhdygan T, Ramirez SH, Prevedel L, Eugenin EA, Al-Harthi L (2020) HIV infects astrocytes in vivo and egresses from the brain to the periphery. In. PLoS Pathog 16(6). https://doi.org/10.1371/journal.ppat.1008381

Maqsood MI, Matin MM, Bahrami AR, Ghasroldasht MM (2013) Immortality of cell lines: challenges and advantages of establishment. Cell Biol Int 37(10):1038–1045. https://doi.org/10.1002/cbin.10137

Mena MA, García De Yébenes J (2008) Glial cells as players in parkinsonism: The "good," the "bad," and the "mysterious" glia. Neuroscientist 14(6):544–560. https://doi.org/10.1177/1073858408322839

Oh J, Schwiebert LM, Benveniste EN (1999) Cytokine regulation of CC and CXC chemokine expression by human astrocytes. J Neurovirol 5(1):82–94

Ottoboni L, Merlini A, Martino G (2017) Neural stem cell plasticity: advantages in therapy for the injured central nervous system. Front Cell Dev Biol 5(May). https://doi.org/10.3389/fcell.2017.00052

Oyefeso FA, Muotri AR, Wilson CG, Pecaut MJ (2021) Brain organoids: a promising model to assess oxidative stress-induced central nervous system damage. Dev Neurobiol 81(5):653–670. https://doi.org/10.1002/dneu.22828

Pandey HS, Seth P (2019) Friends turn foe—astrocytes contribute to neuronal damage in NeuroAIDS. J Mol Neurosci 69(2):286–297. https://doi.org/10.1007/s12031-019-01357-1

Parpura V, Verkhratsky A (2012) Neuroglia at the crossroads of homoeostasis, metabolism and signalling: evolution of the concept. ASN Neuro 4(4):201–205. https://doi.org/10.1042/AN20120019

Placone AL, Mcguiggan PM, Bergles DE, Searson PC, Guerrero-cazares H, Qui A (2015) Human astrocytes develop physiological morphology and remain quiescent in a novel 3D matrix. Biomaterials 42:134–143. https://doi.org/10.1016/j.biomaterials.2014.11.046

Prajapati B, Fatma M, Maddhesiya P, Sodhi MK, Fatima M, Dargar T, Bhagat R, Seth P, Sinha S (2019) Identification and epigenetic analysis of divergent long non-coding RNAs in multilineage differentiation of human neural progenitor cells. RNA Biol 16(1):13–24. https://doi.org/10.1080/15476286.2018.1553482

Priyanka, Wadhwa R, Chaudhuri R, Nag TC, Seth P (2020) Novel role of mortalin in attenuating HIV-1 tat-mediated astrogliosis. J Neuroinflammation 17(1):1–20. https://doi.org/10.1186/s12974-020-01912-3

Rivetti P, Romanov RA, Spigolon G, Masini D, Martín-montañez E, Toledo EM, La Manno G, Feyder M, Pifl C, Ng Y, Sánchez SP, Linnarsson S, Wernig M, Harkany T, Fisone G, Arenas E (2017) Induction of functional dopamine neurons from human astrocytes in vitro and mouse

astrocytes in a Parkinson ' s disease model. Nat Publ Group 35(5):444–452. https://doi.org/10.1038/nbt.3835

Rojas-Celis V, Valiente-Echeverría F, Soto-Rifo R, Toro-Ascuy D (2019) New challenges of HIV-1 infection: how HIV-1 attacks and resides in the central nervous system. Cell 8(10): 1–15. https://doi.org/10.3390/cells8101245

Serramía MJ, Muñoz-Fernández MÁ, Álvarez S (2015) HIV-1 increases TLR responses in human primary astrocytes. Sci Rep 5:1–15. https://doi.org/10.1038/srep17887

Sharma V, Mishra M, Ghosh S, Tewari R, Basu A, Seth P, Sen E (2007) Modulation of interleukin-1β mediated inflammatory response in human astrocytes by flavonoids: implications in neuroprotection. Brain Res Bull 73(1–3):55–63. https://doi.org/10.1016/j.brainresbull.2007.01.016

Siddharthan V, Kim YV, Liu S, Sik K (2007) Human astrocytes/astrocyte-conditioned medium and shear stress enhance the barrier properties of human brain microvascular endothelial cells. Brain Res 1147:39–50. https://doi.org/10.1016/j.brainres.2007.02.029

Singh D, Agrawal A, Singal CMS, Pandey HS, Seth P, Sharma SK (2020) Sinomenine inhibits amyloid beta-induced astrocyte activation and protects neurons against indirect toxicity. Mol Brain 13(1):1–10. https://doi.org/10.1186/s13041-020-00569-6

Takeshita Y, Obermeier B, Cotleur A, Sano Y, Kanda T, Ransohoff RM (2014) An in vitro blood–brain barrier model combining shear stress and endothelial cell/astrocyte co-culture. J Neurosci Methods 232:165–172. https://doi.org/10.1016/j.jneumeth.2014.05.013

Vila M, Jackson-Lewis V, Guégan C, Chu Wu D, Teismann P, Choi DK, Tieu K, Przedborski S (2001) The role of glial cells in Parkinson's disease. Curr Opin Neurol 14(4):483–489. https://doi.org/10.1097/00019052-200108000-00009

Wanner I (2012) An in vitro trauma model to study rodent and human astrocyte reactivity. Methods Mol Biol 814:189–219. https://doi.org/10.1007/978-1-61779-452-0_14

Yan Y, Shin S, Jha BS, Liu Q, Sheng J, Li F, Zhan M, Davis J, Bharti K, Zeng X, Rao M, Malik N, Vemuri M, C. (2013) Efficient and rapid derivation of primitive neural stem cells and generation of brain subtype neurons from human pluripotent stem cells. Stem Cell Transl Med 2:1467–1472

Yuan T, Liao W, Feng NH, Lou YL, Niu X, Zhang AJ, Wang Y, Deng ZF (2013) Human induced pluripotent stem cell-derived neural stem cells survive, migrate, differentiate, and improve neurologic function in a rat model of middle cerebral artery occlusion. Stem Cell Res Ther 4(3):73. https://doi.org/10.1186/scrt224

Index

I. Patro et al. (eds.), *The Biology of Glial Cells: Recent Advances*,
https://doi.org/10.1007/978-981-16-8313-8

O

P

R

S